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Genomic analysis of quantitative disease resistance during maize-hemibiotrophic leaf blight pathogen interaction

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

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DEDICATION

This dissertation and experience is dedicated to my parents Mr. Mustapha Gore, Mrs. Mayee Nakiyini and the late Mr. Sam Kasuzi. I will forever be grateful for the selfless sacrifices they made to ensure that my siblings and I would each have a great education and a life that honored God, elders, and humanity. The person I have become is a reflection of their tireless efforts. May God Almighty bless them abundantly.

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ABSTRACT

Plant diseases account for more than 40% of crop losses. Breeding for disease resistance is the most effective means of crop protection against these losses. Disease resistance in plants is categorized into qualitative and quantitative resistance. Qualitative disease resistance is controlled by single major genes that confer complete or near complete resistance against disease pathogens. However, the absence or instability of qualitative resistance in many crop-pathogen interaction systems has necessitated a shift in research toward quantitative disease resistance (QDR). QDR is conditioned by several genes of minor effects resulting in reduced levels of disease. The multigenic nature of QDR overcomes the limitations of qualitative disease resistance. As a fairly new field of research, the specific mechanisms underlying the genetic architecture of QDR in response to pathogen attack are still not well understood. The goal of this research was to identify and characterize the genetic, molecular and mechanistic bases of QDR in maize against hemi-biotrophic *Setosphaeria turcica*, the causative agent of Northern leaf blight (NLB). The unique lifestyle of *Setosphaeria turcica*, as well as the sequenced and annotated maize genome provide an opportunity to study the quantitative nature of host-hemi-biotrophic interactions.

This research employed both phenotypic and genetic approaches to elucidate the mechanisms that underlie QDR with the aim of improving plant health and production. A new genetic map for the Intermated B73 x Mo17 Syn10 doubled haploid line (IBMSyn10DHL) population was created and evaluated. To identify and characterize the genetic basis of quantitative disease resistance in maize-*S. turcica* interaction, IBMDHLs were tested against two isolates of *Setosphaeria turcica* across multiple environments. Unique and overlapping NLB resistance-related QTL were identified within and across environments respectively.

Additionally, IBMDHL and their backcross hybrid populations were tested in Iowa against Iowa NLB isolate to estimate the genetic mode of action of loci underlying quantitative disease resistance. Results from this research highlighted the multigenic nature and specificity of QDR and the genetic effects of genes underlying QDR. Furthermore, previously reported and novel disease resistance-related QTL were identified.

CHAPTER 1. GENERAL INTRODUCTION AND LITERATURE REVIEW

Maize (*Zea mays*)

By 2050, the human population is estimated to increase to over 9 billion (FAO, 2010). In light of the growing global population, food and feed production particularly of cereals such as maize, rice, and wheat should be increased to meet the growing nutritional demands of humans and animals (http://faostat.fao.org/).

Originally from Mexico and Central America (Piperno 2011), maize (*Zea mays*) is one of the top three cereal crops grown worldwide; United States being the lead producer followed by China and Brazil. Together, these three countries generate approximately 79% of the world's annual maize production (Ranum et al. 2014). In the United States, 38.8% of the maize produced is used as animal feed, 30.5% for ethanol production, and 30.7% for export and human use (www.ncga.com/worldofcorn).

The projected 50% increase in global demand for maize by the year 2020 (Pingali, 2001) highlights the urgent need to mitigate factors limiting its productivity. Maize production is constrained by several abiotic factors including: variable soil characteristics and adverse weather conditions, as well as biotic factors including: pests, pathogens and diseases (Shiferaw et al. 2011). Maize diseases are a major problem, reducing the quantity (yield) and quality of maize grown around the globe. In fact, farmers in the United States alone are estimated to lose 2-15% of maize yield to diseases annually (Yang et al. 2017a). Effects of pathogen and disease stresses on crop production have been exacerbated by climatic and environmental changes, resulting in new diseases, changes in pathogen specificity, and evolution of new forms of pathogens (Boyd et al. 2013).

Foliar diseases are the leading cause of yield losses attributable to diseases in maize. Northern leaf blight is one of the major foliar diseases that has caused devastating yield losses in maize (Krausz et al. 1993; Pratt and Gordon 2006). Genetic resistance is a more effective method to control disease-caused crop losses than cultural, chemical and biological methods (Pilet-Nayel et al. 2017). Genetic and molecular techniques have been used to breed maize lines with improved qualitative resistance, and to identify quantitative disease resistance genes (Kump et al. 2011; Young 1996). Additionally, the diversity of maize phenotypic and genetic architecture, presence of a sequenced and annotated genome, and the ease at which maize can be grown in a broad array of environments, makes it a preferred system to conduct genetic studies (Nannas and Dawe 2015; Strable et al. 2010).

Physical and Genetic Maps

Maize is an essential model organism in scientific research and yet it has one of the most complex genomes (Wei et al. 2009). Sequencing the maize genome is difficult due to maize DNA content being repetitive and the large size of the genome (Wei et al. 2005). However, the maize society has devoted resources towards sequencing maize, and developing and constructing maize physical and genetic maps (Wei et al. 2009). Compared to rice and sorghum whose genomes are 389Mb and 700 Mb respectively (Wei et al. 2005), the maize genome is approximately 2500Mb (Cone et al. 2002). Maize has 10 chromosomes and was first sequenced by (Schnable et al.). Since then, the maize genome has undergone several reassembling stages from B73 RefGen_V1 to the current B73 RefGen_V4 (Jiao et al. 2017; Law et al. 2015; Schnable 2012). B73 RefGen_V1 was sequenced using the bacterial artificial chromosome (BAC-by-BAC) technique, producing a partially ordered and oriented physical map (Wei et al. 2009; Zhou et al. 2009). The present B73 RefGen_V4 has ordered and oriented contigs, and was

assembled using the PacBio Single Molecular Real Time (SMRT) method (Jiao et al. 2017). Assembly and annotation of the maize genome, coupled with integration of physical and genetic maps enabled conduction of evolution, domestication, breeding, genomic and genetic studies aimed at identifying and understanding genetic control of agronomically and economically important traits such as yield, and resistance to pests, diseases, and drought (Cone et al. 2002; Wei et al. 2005).

Setosphaeria turcica

Setosphaeria turcica (syn. *Helminthosporium turcicum*) (Galiano-Carneiro and Miedaner 2017) is an ascomycete hemi-biotrophic vascular fungus that causes Northern Leaf Blight (NLB) in maize and sorghum (Condon et al. 2013; Flaherty and Dunkle 2005; Xue et al. 2013). In the USA alone, the prevalence of NLB has increased from the eastern to the western corn belts devastating large acreages of corn fields (Jackson-Ziems, 2016).

S. turcica is an economically important fungal pathogen can lead to greater than 50% yield losses in maize (Degefu et al. 2004; Ferguson and Carson 2004, 2007; Martin et al. 2011; Poland et al. 2011; Pratt and Gordon 2006; Welz and Geiger 2000; Zhang et al. 2012). Yield losses are exacerbated especially in susceptible hybrids when *S. turcica* infects maize prior to silking and tasseling. Early infection provides the fungus with a longer infection window which culminates in larger leaf tissues being damaged (Jackson-Ziems, 2016).

Cool to moderate temperatures (15-25°C) and high humidity (90–100%) promote the growth of *Setosphaeria turcica* (Galiano-Carneiro and Miedaner 2017). Under these conditions, *S. turcica* spores are transported mainly by wind or splashing rain from the previous season's infected plant residue onto new leaves (Degefu et al. 2004) . *S. turcica* conidia germinate, penetrating maize leaves and or husks through the leaf cuticle and outer epidermal cell walls and

into the cell using secondary hyphae (Degefu et al. 2004). Once inside the cell, *S. turcica* secretes *Helminthosporium turcicum* (HT) toxins killing the cells (Galiano-Carneiro and Miedaner 2017). Cell death results in formation of cigar-shaped lesions that run parallel to the veins on infected maize tissue within approximately 7-12 days of infection depending on the environmental conditions, the host and pathogen (Jackson-Ziems, 2016). These lesions initiate as a few yellowish specs which gradually coalesce forming larger lesions on leaf tissue. Development of lesions advances from the lower canopy to the upper canopy and lesions go from yellow to tan as the growing season progresses (Jackson-Ziems, 2016).

Due to its hemi-biotrophic lifestyle, *S. turcica* needs living host cells for survival during the initial stages of infection (Condon et al. 2013) and later produces HT-toxins that kill the host cells during the necrotrophic developmental stage (Bashan et al. 1995). The latent phase between biotrophic and necrotrophic phases allows for the fungus to go undetected while it establishes itself in the plant.

Host resistance is the most effective control of *S. turcica*. Both qualitative and quantitative forms of resistance against *S. turcica* are available in maize inbred lines (Pratt and Gordon 2006; Welz and Geiger 2000). However, the expression, effectiveness, and durability of qualitative resistance genes such as *Ht1*, *Ht2, Ht3, Htn1, Htm1* and *23N* is limited by evolution of *S.turcica*, race specificity of qualitative resistance, and changes in environmental conditions (Condon et al. 2013; Martin et al. 2011; Xue et al. 2013), Weems & Bradley, 2018). These limitations necessitate the quest for new and durable sources of resistance that are effective against all races of the pathogens in question (Carson and Vandyke 1994; Parlevliet 2002; Welz and Geiger 2000).

Qualitative and Quantitative Disease Resistance

Unlike animals that have both innate and adaptive immunity (Ausubel 2005; Flainik and Du Pasquier 2004; Iwasaki and Medzhitov 2010; Janeway and Medzhitov 2002; Medzhitov and Janeway 2002), plants defend against invading pathogens through innate immune systems alone (Kushalappa et al. 2016; Stael et al. 2015). Under innate immunity, the plants' perception of pathogen associated molecular patterns (PAMPs) induces PAMP-triggered immunity (PTI) (Chisholm et al.), preventing entry of pathogens (Boller and Felix 2009; Macho and Zipfel 2015; Trda et al. 2015). Some examples of PAMPs are found in chitin in fungi and flagella in bacteria. These elicit pattern recognition receptor (PRR) proteins resulting in PTI (Dodds and Rathjen 2010). Some pathogens are able to overcome this basal level of defense, penetrating through plant tissues followed by production of effectors (Oliver and Solomon 2010). Interaction of pathogen effectors with host *R*-genes that encode NB-LRR protein products induces effector triggered immunity (ETI). On the other hand, absence or lack of *R*-genes results in effector-triggered susceptibility (ETS) (Ausubel 2005; Chisholm et al. 2006; Dangl and Jones 2001; Giraldo and Valent 2013; Jones and Dangl 2006). R-gene mediated (qualitative) resistance is deployed by single dominant genes and confers complete or near complete resistance in plants (Kushalappa et al. 2016). *R*-gene mediated hypersensitive response, in which programmed cell death occurs at the point of entry is effective in controlling biotrophic pathogens (Poland et al. 2009). Conversely, this hypersensitive response predisposes the plant to necrotrophic pathogen infection, since necrotrophs thrive on dead cells (Govrin and Levine 2000; Lorang et al. 2007). *R*-gene mediated resistance is therefore less effective against necrotrophs than biotrophs (Glazebrook 2005). Although some level of qualitative resistance has been reported in host plant-

necrotrophic interactions (Johal and Briggs 1992; Leach et al. 2001), quantitative resistance is considered more effective against necrotrophs (Balint-Kurti et al. 2008; Lindhout 2002).

Until recently, crop disease resistance studies have mostly focused on identification and breeding for single gene (qualitative) resistance. However, the absence or instability and isolate specificity of qualitative resistance mechanisms in many crop-pathogen systems has shifted breeding efforts towards QDR research (Burdon et al. 2014; Kover and Cheverud 2007). QDR is controlled by several minor genes that collectively lead to partial disease resistance rather than a complete lack of disease as seen in qualitative disease resistance (Boyd et al. 2013; Burdon et al. 2014; Niks et al. 2015; Poland et al. 2009; Young 1996). The genetic basis of QDR has been attributed to an array of genes involved in several pathways or mechanisms such as basal defense, morphology, developmental regulation, detoxification, defense signal transduction, and unidentified resistance genes (Fukuoka et al. 2009; Krattinger et al. 2009; Kushalappa et al. 2016; Manosalva et al. 2009; Niks et al. 2015; Poland et al. 2009). Consequently, variation in QDR is attributable to a wide range of genetically-controlled mechanisms and pathways (Dunning et al. 2007; Kover and Schaal 2002; Poland et al. 2009). How these various genes, mechanisms, and pathways interact to induce defense at a genetic, biochemical, and molecular level is still minimally understood.

Quantitative resistance in maize against NLB pathogens is controlled by alleles at several functional polymorphisms (Balint-Kurti et al. 2010; Balint-Kurti et al. 2007; Chung et al. 2010; Kump et al. 2011; Kump et al. 2010; Wisser et al. 2008). The study by (Kump et al. 2011) revealed that the functionality of detoxification pathways, the major player in maize defense against blight pathogens was determined by polymorphism of an amino acid in a glutathione-S-transferase. However, it is still challenging to achieve a comprehensive mechanistic

understanding of these disease reducing genetic loci. Additionally, not much is known about the genetic effects of the alleles associated with QDR traits. Investigating the genetic bases of QDR in both inbred and hybrid backgrounds, and exploring the impact of QDR on leaf blight pathogenesis will broaden our understanding of plant-pathogen interactions and aid our efforts to evaluate some of the hypotheses that have been reviewed by (Kou and Wang 2012; Poland et al. 2009; St Clair 2010) as the bases of QDR.

Genotype by Environment Interaction

Breeding for disease resistance is the most economical and effective method for controlling diseases in plants. Qualitative and quantitative resistance genes have been identified in multiple crops, including rice (Xue-Wen Xie et al., 2008), maize (Pratt and Gordon 2006; Welz and Geiger 2000; Yang et al. 2017b), and wheat (Buerstmayr et al. 2009; Liu et al. 2009). Variation in phenotypic traits is affected by genotype, environment, and genotype by environment interaction (Moose and Mumm 2008). The dependence of phenotypic expression of genetically controlled traits on the environment has undermined breeding efforts for resistance (Johana et al. 2017; Li et al. 2018; Mukherjee et al. 2013). Therefore, it is important to study the impact of genotype-by-environment (G x E) interaction on QTLs controlling phenotypic traits in plants under multiple environments (Drake-Stowe et al. 2017; Paterson et al. 1991; Stuber et al. 1992; Young 1996).

Differences in disease resistance are brought about by variation in environmental conditions and pathogen isolates/races. These factors impact the type and level of disease resistant genes that are elicited which in turn impacts disease resistance phenotypes (Asins 2002; Nelson et al. 2018; Yang et al. 2017a). Host-pathogen interactions determine the scope of effectiveness of existing resistant genes. Isolate non-specific QTLs are effective against a wide

continuum of pathogen isolates/races while isolate specific QTLs are effective against a few isolates/races (Pilet-Nayel et al. 2017). In light of this, geneticists and breeders have been charged with the task of testing germplasm in varying environments to identify QTLs that are stable across environments (Johana et al. 2017) and are isolate/race non-specific.

Gene Effect in Different Maize Populations

The multigenic (quantitative) control of disease resistance and impact of genetic and environmental factors on expression of resistance makes genetic improvement in plants challenging. Therefore, creation of populations with superior genetics that are stable across various environments is crucial (Hallauer et al. 2010). Biparental mating, polycross, North Carolina design I,II,III and diallel cross are the six main categories of mating designs that breeders use to produce high quality diverse populations in maize (Acquaah 2007; Hallauer et al. 2010). Biparental is the simplest mating design and involves crossing pairs of randomly selected individuals to produce 1/2n full-sib families. This design is complicated by its inability to provide genetic and environmental variance estimates because the only sources of variation come from within and between families. Under the polycross design, groups of plants that can only undergo cross-pollination are randomly intermated. A successful polycross needs synchronized flowering across all the plants involved in the cross and pollen to be randomly distributed across plants within a given experimental block (Acquaah 2007; Hallauer et al. 2010). However, this is hard to achieve. North Carolina I (NCDI) is the most commonly used mating design because of its ability to estimate both half- and full-sib selection and genetic variance in self and cross-pollinated individuals and in animals (Acquaah 2007; Hallauer et al. 2010). NCDI involves mating independent males to different groups of females which restricts estimation of genetic variance components to the specific population being tested (Acquaah

2007). Unlike NCDI, independent males are crossed with the same group of females in North Carolina design II and this improves precision (Acquaah 2007; Hallauer et al. 2010). Variances due to male, female, male-female interaction and within family can be calculated from the NCDII analysis of variance (ANOVA) table. Under the North Carolina design III (NCDIII), F_2 plants are randomly selected and crossed back to their inbred parents. Estimates of genetic variance components are determined under the assumption that epistasis and linkage are not present. Lastly is the diallel cross, which not only encompasses self but also reciprocal crosses (Acquaah 2007; Hallauer et al. 2010). These mating designs provide breeders and geneticists with genetically diverse populations (resources) from which gene actions underlying quantitative trait loci can be assessed.

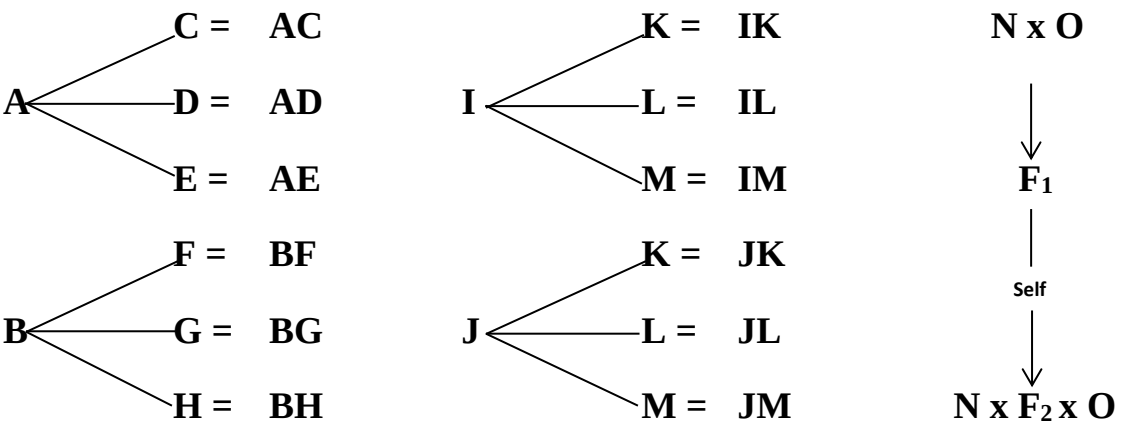

Figure 1: Mating designs; North Carolina Design I, North Carolina Design II and North Carolina Design III.

Research Objectives

The overall goal of the research projects was to broaden and deepen our understanding of the genetic mechanisms underlying quantitative disease resistance during host-pathogen interactions. A panel of Intermated B73 x Mo17 doubled-haploid lines (IBMDHLs) and their back cross hybrids was screened against hemi-biotrophic *Setosphaeria turcica.* Advancement in our knowledge of plant host-pathogen interactions will inform breeding choices for effective resistance. The major objectives were:

1. Construct and evaluate a new genetic map for the intermated B73 x Mo17 Syn10 doubled haploid line (IBMSyn10DHL) population
    - To anchor unanchored contigs in the B73 physical map
2. To identify and characterize the genetic basis of quantitative NLB resistance in the IBMDHL populations
    - To screen a panel of Intermated B73xMo17 doubled haploid lines (IBMDHLs) for resistance to *Setosphaeria turcica*
    - To identify quantitative northern leaf blight resistance loci in an IBMDHL population
    - To determine the specificity of identified quantitative northern leaf blight resistance loci
3. To Identify and characterize the genetic mode of action of quantitative NLB resistance in a hybrid context by leveraging parental backcross IBMDHL populations
    - To assess gene actions underlying identified resistance QTLs in inbred and backcross populations

Rationale and Significance

With the predicted 9.1 billion person increase in human population (Yang et al. 2017a), and 60-110% increase in demand for agricultural production by 2050, controlling crop losses due to diseases and pests by breeding for durable resistance has become mandatory (Mundt 2014). Maize is the third most valuable cereal crop in the world after wheat and rice (Ullstrup 1972) and yet its production is still largely constrained by diseases that result in reduced yield and grain quality (Yang et al. 2017a). Of great economic value is *Setosphaeria turcica,* the causal agent of Northern leaf blight (Krausz et al. 1993). In the past, *Setosphaeria turcica* has reduced maize yield by 50% and still has great potential to adversely reduce maize yields if not controlled (Degefu et al. 2004; Ferguson and Carson 2004, 2007; Martin et al. 2011; Poland et al. 2011; Pratt and Gordon 2006; Welz and Geiger 2000; Zhang et al. 2012). The magnitude of yield loss is influenced by properties of the pathogen, host, and environment.

Breeding for host resistance is the most effective and reliable means of protecting plants against pathogens. Single genes (qualitative) confer complete resistance while multiple genes with minor effects confer incomplete resistance (quantitative) (Li et al. 2018). The loss of durability of major *R*-genes was demonstrated in the *S. turcica* race distribution study conducted by Weems & Bradley (2018). On average, 33% of the 156 NLB isolates (20 physiological races) collected from seven states caused disease in the presence of *Ht1, Ht2, Ht3, Htn1* and *Htm1* genes. Genetic and molecular studies have shown quantitative resistance to be more durable and in some plant-pathogen interactions the only form of resistance (Castro et al. 2003; Niks et al. 2015; Pariaud et al. 2009; Poland et al. 2009). However, the multigenic nature of quantitative resistance hinders fast-paced production of superior genotypes. The small effects contributed by the minor genes that underlie QDR further complicate identification of resistance genes.

Additionally, genotype, environment, and the genotype-by-environment (G x E) interaction influence phenotypic expression of QTLs (Li et al. 2018).

Understanding the genetic effects of genes underlying quantitative trait loci (QTL) is core to improving quantitatively controlled traits (Li et al. 2010). Moreover, most genetic and breeding research on inheritance of quantitative traits is carried out mainly in inbred lines (Jamann et al. 2014; Kump et al. 2011; Poland et al. 2011; Zila et al. 2014). Effectiveness of inbred derived data in predicting hybrid performance can be improved by assessing gene actions in genetically diverse populations. Understanding the implications of additive, dominant or overdominant, additive by additive, additive by dominant and dominant by dominant (Hallauer et al. 2010) genetic effects on disease resistance in both inbreds and hybrids will provide valuable information needed to select resistant parents for breeding (Bernardo 2002).

It is also important to integrate physical and genetic maps to provide a comprehensive and accurate resource for identifying and functionally characterizing genes involved in QDR and assessing environmental effects on their expression. These resources will broaden our understanding of the complex nature of QDR relative to plant-pathogen interactions. Results from our study will provide additional invaluable sources of durable resistance to breeding programs.

Dissertation Organization

Setosphaeria turcica is still a threat in the maize producing communities around the globe. As such, identifying durable resistance against this pathogen is vital. This dissertation consists of four chapters written in journal paper format.

Chapter one is an introductory section that gives a brief background of the host (maize), the pathogen (*Setosphaeria turcica*), the varying mechanisms employed by maize to resist

infection by *Setosphaeria turcica* and the different factors affecting maize-*Setosphaeria turcica* interaction. Within this chapter, I also cover what is already known about control of *Setosphaeria turcica*, the knowledge gaps that still exist, and how the dissertation projects will address these gaps.

Chapter two addressed the need to have a comprehensive reference genome from which genes underlying quantitative disease resistance loci are identified. Genome-wide single nucleotide polymorphism (SNP) IBMDHL sequences were physically mapped, followed with construction of a physically-ordered genetic. The IBMDHL population provided us with a high marker density map and high resolution for marker identification. The work in this paper resulted from collaborative efforts between Pioneer-HiBred and Dr. Nick Lauter's laboratory (USDA-ARS & Department of Plant Pathology and Microbiology, Iowa State University). Pioneer-HiBred conducted Illumina sequencing of the 332 IBMDHLs. Various members of the Lauter Laboratory contributed to construction and evaluation a new genetic map which I have described in chapter two under the guidance of Dr.Nick Lauter.

Chapter three made use of the maps constructed in chapter two to identify quantitative trait loci (QTL) associated with resistance to *Setosphaeria turcica*. IBMDHL population was provided by Pioneer-HiBred and were screened for resistance to *Setosphaeria turcica* in manually inoculated fields located in Aurora, New York and Boone, Iowa. Disease severity scores (phenotypic data) were collected from the IBMDH lines and used for QTL analysis. Quantitative Northern leaf blight resistance (NLB) loci common to both locations or unique to the individual locations were identified across the IBMDHL population. This work was a collaborative effort between Pioneer-HiBred, Dr. Rebecca Nelson's laboratory (Department of Plant-Microbe biology, Cornell University), Dr. Nick Lauter's laboratory (USDA-ARS &

Department of Plant Pathology and Microbiology, Iowa State University), and Dr. Alison Robertson's laboratory (Department of Plant Pathology and Microbiology, Iowa State University). Pioneer-HiBred developed and provided the IBMDHL population and sequenced the lines using Illumina technology. Field experiment activities included NLB inoculum production, preparation of the IBMDHL seed, planting, inoculation and phenotypic data collection. Dr. Judith Kolkman and Dr. Rebecca Nelson's laboratory members executed the Aurora, NY field experiments. I executed the Boone, IA field experiments plus QTL analysis with the help of members in Dr. Alison Robertson's and Dr. Nick Lauter's laboratory groups. All the research was conducted with and under the guidances of Dr. Nick Lauter and Dr. Alison Robertson.

Chapter four focuses on assessing the impact of gene effects on genes underlying Quantitative Northern leaf blight resistance (NLB) loci. The three maize populations (IBMDHL, BC_{B73} and BC_{Mo17}) were developed and provided by Pioneer-HiBred, planted at the Agricultural Engineering and Agronomy Research farm in Boone, IA, manually inoculated with *Setosphaeria turcica* and screened for resistance to *Setosphaeria turcica*. The two backcross hybrid populations were developed following the North Carolina mating Design III by crossing IBMDHLs to their parental lines B73 and Mo17. Shared and unique quantitative Northern leaf blight resistance (NLB) loci were identified across the three maize populations. Additive, dominant and codominant gene effects were identified across the three populations. Similar to chapter three, I executed the field activities and QTL analysis with the help of members in Dr. Nick Lauter's laboratory group. All the research was conducted under the guidance and training of Dr. Nick Lauter and Dr. Alison Roberston.

Chapter five summarizes the main outcomes and conclusions of the above mentioned projects and describes the significance of these outcomes in the breeding and genetics research world.

Secondary Research Projects

In addition to the projects described above, I was part of a collaborative project that assessed the molecular mechanisms underlying genes that confer resistance to multiple diseases. This project involved Dr. Peter Balint-Kurti's laboratory group at North Carolina State University & USDA, Dr. Nick Lauter's laboratory group, Iowa State University & USDA-ARS, Dr. Jeffrey Caplan & Dr. Randall Wisser's laboratory and group at University of Delaware, Judith Kolkman & Dr. Rebecca Nelson's laboratory group at Cornell University and Dr. Michael Kolomiets at Texas A&M University. Under the guidance of Dr. Nick Lauter, Mercy K. Kabahuma conducted transformation using agrobacterium and provided agrobacteria containing our genes of interest to Dr. Kan Wang's laboratory group at the Plant transformation facility, Iowa State University to infect maize embryos. Mercy K. Kabahuma managed transgenic plants from plantlets to seed production in the USDA-ARS greenhouse at Iowa State University. The detailed description of the background, methodology, results, conclusions, and all of the collaborators' contributions to this project can be found in the paper published in Nature Genetics entitled "**A gene encoding maize caffeoyl-CoA O-methyltransferase confers quantitative resistance to multiple pathogens**"

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CHAPTER 2. INTEGRATING MAIZE GENETIC AND PHYSICAL MAPS USING UPDATED RESOURCES

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Abstract

The maize genome is large and complex, comprising “oceans of repetitive DNA” surrounding “gene islands” of variable sizes and gene densities. The genome is also dynamic, experiencing approximately one recombination event per chromosome arm per meiosis and accumulating new variants in sequence and gene copy numbers at a relatively high rate, offering a wealth of substrate for evolution and breeding to act upon. A long-standing and ongoing challenge is to construct a detailed knowledge base of functional variation across the complex and dynamic gene space. An important foundation for this pursuit is a complete physical map and a direct integration of that physical map with a high-resolution genetic map. Construction of this foundation has been ongoing for several decades, focusing on inbred line B73 for the

reference physical map, with genetic contrasts to more than 25 other inbred lines. Recently, the physical map for B73 was re-assembled using new technologies, solidifying the physical side of the interplay between genetic and physical genome mapping. Here we report the integration of the new physical map for B73 with a high resolution genetic mapping resource, the Intermated B73 x Mo17 Synthetic-10 Doubled Haploid Line (IBMSyn10DHL) population. During this integration, non-parental haplotypes were observed among the IBMsyn10DHLs, requiring data masking of ~2.5% of the genotype data to permit appropriate utilization of bi-parental statistical frameworks for genetic analysis. The product of this work is a genetic map built from 4,191 single nucleotide polymorphisms (SNPs) genotyped for 247 IBMSyn10DHLs, which captures the complete genetic space in 1854.1 cM. Each SNP occupies a unique genetic position and is physically anchored, allowing direct and precise projection of genetic results onto the physical map, a critical process which has been a barrier to mapping populations in the past.

Key words: Intermated B73 x Mo17 Synthetic-10 Doubled Haploid Line population, single nucleotide polymorphisms

Background

Maize is one of the most important crops grown around the globe mainly for food, feed and fuel (Hake and Ross-Ibarra 2015; Ranum et al. 2014). The value of maize is heightened by a 60% projected increase in demand (http://bdspublishing.com/_webedit/uploaded-files/All%20Files/Maize%20blog%20key%20challenges.pdf) for feeding the growing global population, which is projected to reach 9.8 billion people by 2050 (UN DESA report, 2017). Dissection of the phenotypic consequences of natural genetic variation is required for understanding the utility and feasibility of manipulating agriculturally important traits. Identification, characterization and cloning of natural quantitative trait locus (QTL) variants is

required to enable this approach, and relies among other things on comprehensive and precise genome sequence (Ashikari and Matsuoka 2006; Guo et al. 2010; Kujur et al. 2016; Ogut et al. 2015; Wang et al. 2018). The advent of improved technologies has enabled sequencing and assembly of complex eukaryotic genomes of several species, including human (Lander et al. 2001; Yu et al. 2001), yeast (Tong et al. 2004), rice (Eckardt 2000), sorghum (Paterson et al. 2009), wheat (Brenchley et al. 2012), soybean (Schmutz et al. 2010) and maize (Schnable et al. 2009; Tong et al. 2004; Yu et al. 2001). For advancing the dissection of natural variation in maize, the availability of assembled genomic sequences has shifted the burden back to development of high resolution mapping populations and the integration of their genetic maps with the assembled genomes.

As a crop species and a biological model for plant genetics and genomics, maize occupies a unique research space in which the public and private sectors have invested, and continue to invest (Hake and Ross-Ibarra 2015). Over the past 50 years, the Iowa Stiff-Stalk-Synthetic line B73 has been the most-widely utilized inbred line for building genetically immortalized resources, in part because it was an important foundational line used in early single-cross hybrid commercial breeding. As such, B73 has been incorporated into public maize genetics resources to enable functional biological studies, most notably the Intermated B73 x Mo17 synthetic-04 Recombinant Inbred Line (IBMsyn04RIL) population (Lee et al. 2002), and as the common parent of the Nested Association Mapping (NAM) population (Yu et al. 2008). On the physical side, B73 was selected as the initial maize reference inbred for genome sequencing and assembly (Schnable et al. 2009; Wang et al. 2016). These resources collectively provided early opportunities for integration of physical and genetic maps, which in many cases were undertaken to leverage the deep knowledge of genetic space for obtaining a more correct order of physical

chunks of sequence whose exact order and orientation remained unclear (Bauer et al. 2013; Coe et al. 2002; Fu et al. 2006; Ganal et al. 2011; Sharopova et al. 2002; Wei et al. 2009).

The maize B73 genome is estimated to contain ~2.3 Gbp and currently includes ~39,324 high confidence gene models which are the product of innovative molecular and bioinformatic technologies (Jiao et al. 2017; Law et al. 2015; Schnable et al. 2009; Wang et al. 2016). However, the path to this point has been arduous and non-linear. The first maize reference genome (Schnable et al. 2009) was assembled by The Maize Genome Sequencing Consortium using bacterial artificial chromosomes (BACs) as substrate. Approximately 16,000 BACs in a putative minimum tiling path (Wei et al. 2009) were used to sequence and assemble the initial B73 genome, resulting in 435 anchored pseudo-contigs and approximately 350 unanchored pseudo-contigs covering an estimated 93% of the genome (Wei et al. 2009; Zhou et al. 2009). B73 RefGen_v2 and B73 RefGen_v3 were based on incremental improvements in the underlying BAC tiling path of B73 RefGen_v1, as well as some incremental improvements in predicting the gene space (Law et al. 2015). However, neither of these versions treated the fundamental weakness in the initial genome sequence and assembly, which was that on average, each the ~16,000 BAC sequences were themselves an assemblage of ~8 unordered and unoriented sequences (Wei et al. 2009; Zhou et al. 2009). Thus, the size of the ~130,000 *true sequence* contigs was ~18,000bp, and their order and orientation within each BAC of approximately 140,000bp was unknown. Although this sounds bleak, high-content-fingerprinting approaches had been used to assemble the 16,000 BACs into only several hundred pseudo-contigs whose boundaries and orientations were nearly entirely correct, making the B73 RefGen_v3 a remarkable resource that greatly advanced maize genetics and genomics. However, the limitation

of BAC-level physical resolution presented a significant obstacle to QTL cloning, which requires detailed and empirically accurate integration of physical and genetic spaces.

Recently, the sequencing and assembly of B73 RefGen_v4 (Jiao et al. 2017; Wang et al. 2016) was completed and represents a significant improvement over prior versions due to a 52-fold increase in true sequence contig length as well as an enrichment of the centromeric and intergenic sequence assembly. B73 RefGen_v4 was sequenced using PacBio Single Molecular Real Time (SMRT) reads and *de novo* assembled using hybrid assembly scaffolds that included optical mapping data, resulting in the dramatic increase in genome contiguity. Although there are 54 small contigs that are not yet anchored and 2,522 small gaps in the assembly, B73 RefGen_v4 has more than sufficient accuracy and detail to support genetic finemapping, provided that high resolution genetic resources are connected to the assembly.

The Intermated B73 x Mo17 synthetic-10 Doubled Haploid Line (IBMsyn10DHL) population was produced through 10 generations of random intermating to increase genetic resolution, followed by haploid doubling to achieve genetic immortality of the bi-parentally derived lines (Hussain et al. 2007). The genetic contrast between the B73 and Mo17 genomes has been captured in several prior public-sector research populations of increasing genetic resolution, including an F_2RIL population (Stuber et al. 1992) and the aforementioned IBMsyn04RIL population (Lee et al. 2002). Although the genetic contrast spans a heterotic divide between the Iowa Stiff Stalk Synthetic and Lancaster Non-Stiff Stalk pools and thus is not viewed as a line development cross, these populations have been extensively utilized by geneticists for discovery and dissection of agronomically important traits (Hu et al. 2016; Jansen et al. 2015; Liu et al. 2015b; Ma et al. 2018; Poland et al. 2011; Silva et al. 2018; Zhao et al. 2018). The widespread use of the B73 x Mo17 intermated resources is likely due both to the

direct connectivity with the B73 genome and the high genetic resolution that is achieved by intermating. Interestingly, the high degree of intermating in the IBMsyn10DHL population likely captures more experimentally-derived recombination events per line than any other immortalized eukaryotic population (Hussain et al. 2007; Liu et al. 2015a; Ma et al. 2018). However, there has not yet been a careful examination of the uniqueness and physical distribution of these cross-over events, nor the levels of genetic detail required to unveil them, nor the relative gains in genetic resolution that they may afford to experimentalists. To begin to address these knowledge gaps, this study tackles the challenge of creating a new genetic map for the IBMSyn10DHL population and integrating it with the B73 RefGen_v4 physical map.

Materials and Methods

Plant materials and relationship to IBMsyn10DHL utilization by other investigators

On behalf of Pioneer-HiBred International, Hussain and colleagues (2007) deposited 360 IBMsyn10DHLs at the USDA-ARS Maize Genetics Cooperative Stock Center (MGCSC). However, seed quantities were not sufficient to allow widespread distribution, thus, no stock ordering information for the lines is available at present. Dr. Michael Lee (Iowa State University), who had already been integral in developing the IBM resources (Lee et al. 2002; Sharopova et al. 2002), obtained the only IBMsyn10DHL seed distribution made by the MGCSC (personal communication with Marty Sachs, MGCSC Director). This distribution is the source of several quantitative genetic analysis publications utilizing the resource by Dr. Lee and collaborators (Jansen et al. 2015; Liu et al. 2015a; Liu et al. 2017; Liu et al. 2015b; Ma et al. 2018; Silva et al. 2018; Trucillo Silva et al. 2017; Zhao et al. 2018). Each of these studies utilizes the genetic map constructed by Liu and colleagues (Liu et al. 2015a), which is based on a genotyping by sequencing approach followed by imputation. Separately, Dupont-Pioneer

scientists utilized the IBMsyn10DHL resource to perform an expression QTL study (Holloway et al. 2011). For the present study, seed stocks for 390 IBMsyn10DHL lines were obtained directly from Pioneer-HiBred International, as facilitated by Dr. Sara Lira. Using the nomenclature provided to us by Pioneer-HiBred International, the lines are all named SX19syn10DH_MXXXX, where the final four characters are non-consecutive numbers ranging from 0001 through 0647 (Table 1). In these names, IBM is replaced by SX19, which was the original name of the commercial hybrid made from the B73 x Mo17 cross. Because "Intermating" is implied by the breeding term "synthetic" and the progeny of a hybrid are the synthetic-01 generation, this nomenclature is ideal and is retained for this study when referring to individual lines, which protects the original provenance of their names assigned by Pioneer-HiBred International. Unfortunately, these DHL names do not connect to any line names used in the above-listed literature; Holloway et al. (2011) provides no list of their 135 DHLs used and no genotype data, and the other papers each cite Liu and colleagues (2015a), which uses 280 lines with names in the form of IBM_XXX, where the final three characters are non-consecutive numbers ranging from 001 through 328. At a minimum, future inter-connectivity could be established via the genome-wide SNP data sets using a coordinate-based approach.

Genome-wide Single Nucleotide Polymorphism data acquisition

The Illumina MaizeSNP50 BeadChip (Ganal et al. 2011) was used by Pioneer-HiBred International to collect genome-wide SNP data (56,110 assays) for 356 IBMsyn10DH lines. Data were made available to this project by Dr. Jonathan Ho and Dr. Sara Lira for use in refining genetic map construction and for purposes of quality control. Standard data quality metrics were applied to produce the genotype calls distributed to the project. There are 414 unique

SX19syn10DH_MoXXX identifiers across the seed and SNP materials received, with an intersection of 332 IBMsyn10DH lines (Table 1).

Single Nucleotide Polymorphism data acquisition for quality control

To be certain that the seed stock and SNP data were correctly interconnected, a quality control set of 36 SNPs were designed and assayed using the iPLEX Gold chemistry detected on a Sequenom Instrument (Agena BioScience, San Diego, CA). Data were collected for 384 of the lines corresponding to the original 390 seed stocks we received (Table 1). The assay was designed such that four genetically linked SNPs at each of nine loci were assayed, providing intrinsic reliability measures within the SNP data (Table 2). The SNPs targeted by the assay were chosen from the set targeted by the Illumina MaizeSNP50 BeadChip so that they would be directly cross-comparable as a quality control measure.

Physical mapping of genetic marker-associated sequences

BLAST (Altschul et al. 1990) was used to determine the physical positions of the 56,110 SNPs in the B73 RefGen_v4 physical map. An e-value of less than 1e-10 was required to retain positional data, such that data from 568 SNPs were not retained. Notably, these may align to a future physical map of B73 as remaining gaps are closed. It is also possible that the SNPs were derived from maize sequences that are absent from B73 (Springer et al. 2009). In the initial output, positions were retained for the top four BLAST hits corresponding to each query. Comparison of the e-values among the top two hits for each query revealed that the design sequences for 301 of the SNP assays match more than one location in the genome equally well, and were thus removed from the genotype data set (Table 3).

Construction of a physically-ordered genetic map

The advent of B73 RefGen-v4 physical map makes possible as shift in how genetic and physical spaces are integrated for maize. Previously, genetic data were utilized to order physical fragments, serving as an empirical benchmark (Coe et al. 2002; Wei et al. 2009). In the present study, we leverage the B73 physical map as an empirical benchmark for two reasons. First, the new physical data are more reliable than ever (see above); second, the intrinsic complexity of genetic data obtained from intermated doubled haploid materials can lead to widespread mis-ordering of genetic markers at the local level. Thus, the SNP data were ordered by the outcome of physical positioning and used as input for genetic distance computation using Joinmap 4.0 (Kyazma Genetics, The Netherlands). The intermating generations in the breeding scheme were accounted for (I=9) in the calculation of inter-marker distances such that the resultant estimates of centiMorgan (cM) distances are not grossly inflated. As such, they are intended to represent Haldane (Collard et al. 2005) recombination-based units arising on a per meiosis basis.

Iterative steps of data filtering followed by map reconstruction were imposed at the line and SNP levels. For line filtering, these steps included removal of lines that failed line-identity quality control, had more than 10% missing genotype data, or were identical to other lines in the population. For SNP filtering, monomorphic SNPs were eliminated first. Next, segregation distortion was evaluated to identify SNPs that were "artificially polymorphic", meaning that the minor allele occurred in such low frequency that it was likely to be a genotyping error. A subset of such SNPs were examined in detail and found to be monomorphic between B73 and Mo17, validating the choice to remove them from the data set. Filtering based on genetic binning was then imposed; this process was conducted using Rqtl (Broman et al. 2003) to identify markers that do not differ from one another and thus must be reduced to one marker to represent each

unique genetic position, referred to as a bin. The bin markers retained were those with the smallest amount of missing data. These processes were then reiterated, since the outcomes of one filtering method can render a new data set that requires filtering based on a different criterion.

Results and Discussion

This study undertakes the task of creating a new genetic map for the IBMSyn10DHL population and integrating it with the B73 RefGen_v4 physical map, which is intended facilitate accurate mapping of quantitative traits while retaining maximal precision. The results below explain and justify the steps taken to achieve the end products, the quality and utility of which are discussed as they are presented. Beyond the routine presentation of genetic map construction outcomes, a key issue tackled by this study is the discovery and treatment of non-parental haplotypes segregating in the IBMsyn10DHL resource, a troubling finding that calls into question the fidelity of prior results obtained using the resource, and therefore threatens the utility of the resource for research. An initial solution that retains the bi-parental analysis framework is presented.

Initial SNP and DHL filtering outcomes

Beginning with 55,126 SNPs for 332 lines for which seed stocks were in hand, 17,062 SNPs were eliminated due to monomorphism or unreliability due to insufficiently low levels of missing data. One line was also removed because seed could not be propagated, yielding a data matrix of 38,064 x 331. Then, 49 addition DHLs were removed due to greater than 10% missing data, which included heterozygous genotype calls that had been removed, such that they appeared equivalent to a failure to make a genotype call. Based on the removal of these lines, an additional 639 monomorphic SNPs were removed. Then, 1,216 SNPs with greater than 3%

missing data were removed, followed by removal of 278 SNPs for which no physical coordinates were obtained, yielding a data matrix of 35,931 x 282.

At this stage, a graphical display of genetic marker data was constructed in order to visually examine pattern in the data. A striking observation was made; large numbers of SNPs showed high levels of segregation distortion, with minor allele frequencies at distorted markers so low that it suggested an error of some type (Fig. 1).

Confirmation of non-parental haplotypes segregating among IBMsyn10DHLs

Examination of cases of segregation distortion at SNPs indicated that they were "artificially polymorphic", or not the result of an actual polymorphism between B73 and Mo17 (Fig. 1). This problem appeared to have genetic, rather than technical underpinnings, because the distorted markers appear in local clusters and because the "minor alleles" are carried in linkage blocks in small subsets of DHLs (Fig. 1). These attributes suggest that the "artificial polymorphisms" are the manifestation of a non-parental haplotype as it would be detected by the binary SNP calling biochemical and bioinformatics procedures used by Illumina's BeadChip genotyping platforms. Since both B73 and Mo17 genomes have been sequenced and assembled (Jiao et al. 2017; Sun et al. 2018), we were able to discern that such "polymorphic" SNPs are actually invariant between B73 and Mo17, and therefore must represent non-parental DNA.

Removal of artificially polymorphic markers

Acting on the new knowledge of non-parental haplotype presence, SNPs with a minor allele frequency lower than 0.07 were marked and tabulated, allowing removal of 35 DHLs with >2% non-parental alleles by this measure. This reduction to 247 DHLs resulted in identification of 3,873 additional monomorphic markers to be removed. To treat the issue of artificial

polymorphisms, SNPs with a minor allele frequency lower than 0.07 were then removed, yielding a data matrix of 24,717 x 247.

Filtering based on genetic binning was then imposed to remove 17,885 SNPs that do not identify additional cross-over events, yielding a data matrix of 6,832 x 247 (see Materials and Methods). The removal of 17,885 artificially polymorphic SNPs underscores the importance of the discovery of non-parental haplotypes. A visual comparison of the graphical representations of the genotype data sets before and after these removals is striking (Fig. 1). The data matrix of 6,832 x 247 was used for genetic map construction, which revealed an additional 87 SNPs whose genotype data did not match the genetic position. Many of these were among the list of assays that query more than one genomic location (Table 3), which accounts for their apparently poor data quality. Map construction proceeded using a data matrix of 6,745 x 247, the results of which are discussed below.

Removal of non-parental haplotype data from the genotype data set

To allow correct usage of bi-parental statistical analysis frameworks for this population, we undertook a process of masking regions of the genome within each line which appeared to harbor non-parental haplotypes. Unfortunately, the removal of artificially polymorphic markers detailed above does not actually remove the contaminating haplotype data, which can be plainly visualized even after artificially polymorphic SNPs are removed (Fig. 1). Although the obvious stretches of contamination remain visible in a graphical display, where they appear as a "barcode" of improbably high recombination rate per Mbp, it is not possible to declare all of these regions as contaminated with non-parental haplotypic DNA. Thus, we utilized a manual masking strategy that leveraged the presence of artificial polymorphisms to specify which regions should be converted to missing data (see Materials and Methods). In total 52,867

genotypic data points (3.17%) were masked in the 6,745 x 247 data set, which contained 1,666,015 data points, 555 of which were missing even prior to masking. Fig. 2 graphically displays the entire unmasked and masked versions of the 6,745 x 247 data set.

Following masking, filtering based on genetic binning was then imposed to remove 2,554 SNPs that do not identify additional cross-over events, yielding a data matrix of 4,191 x 247, which was used for genetic map construction. In this matrix, there are only 29,031 missing data points (2.80%) from a total of 1,035,177 data points. The reduction in number of missing data points occurred during the removal of the 2,554 SNPs whose formerly unique bin positions were collapsed when the contaminating haplotypic data were removed.

Comparison of genetic maps built from unmasked and masked data

The masked map is dramatically reduced in length, resulting from the removal of data points that falsely exaggerate genetic distance per Mbp of physical genetic space (Fig. 3, Table 4). Indeed 2,554 non-bin marker SNPs were removed following data masking of non-parental data, leading to a 46.5% reduction in map length, corresponding to 1610.8cM. Several attributes of the masked map suggest that it will provide improved performance for QTL mapping compared to the unmasked map. Above all, the average intermarker distance is smaller, 0.4cM compared with 0.5cM (Table 4). This can also be seen in smaller maximal intermarker distances for each of the ten chromosomes, again indicating that performance for QTL mapping will be improved (Table 4). Future tests of performance of these two maps during QTL analysis will be required to determine actual levels of improvement in precision and accuracy that are likely to arise from employment of the “decontaminated” data set.

Comparisons to the best published genetic map for the IBMsyn10DHL resource

Liu and colleagues (2015a) published a deeply resourced and very carefully constructed map using 280 IBMsyn10DHLs. The map produced in the study was constructed from 6,618 markers residing in unique genetic bins, a number that is remarkably close to the 6,745 bin markers identified by our study. Of interest is the fact that Liu and colleagues drew upon 1,151,856 SNPs to produce their map, while ours was constructed from only 55,126. Despite the disparity between numbers of DHLs used (280 versus 247), an overall conclusion of this observation is that both efforts saturated the extant genetic cross-over events with more than adequate levels of genetic detail; one map retained 0.57% of the polymorphic SNPs, while the other retained upwards of 40% of the truly polymorphic SNPs. This finding is consistent with that of genetic maps built for the IBMsyn04RIL resource, which suggested the existence of only a commensurate density of genetic bins when adjusted for population sizes and numbers of generations of intermating (Fu et al. 2006; Sharopova et al. 2002). The effectiveness of the Illumina MaizeSNP50 BeadChip should also be given its due credit, since the SNPs assayed by the platform were chosen from a similarly large set of known polymorphisms to deliberately cover the genome with maximal dispersion (Ganal et al. 2011).

At a glance, it is curious that Liu and colleagues (2015a) did not detect any non-parental haplotype signatures, despite their deeply resourced and very carefully executed efforts. However, we surmise that this failure of detection occurred through a series of deficiencies that were embedded in the technologies and resources that were applied at the time their study was conducted. Foremost, detection of tri-allelic SNPs or non-parental bi-allelic SNPs using a genotyping by sequencing approach based on Illumina reads would best be achieved through deliberate searches, because standard data quality filters would generally remove the data that

would reveal such non-parental contamination. There would have been no reason for them to conduct such searches, and if they had, it is unclear that the potential signals could have statistically risen above the noise. Equally important was the constraint imposed by quality limitations of the physical map at the time they conducted the work. Without B73 RefGen_v4 (Jiao et al. 2017), SNPs were statistically ordered using advanced genetic mapping approaches to deal with the high volume of data. Indeed, a graphical examination of the Liu et al. (2015a) genotype data set reveals no striping or barcoding. As described above, striping was likely averted through the use of SNP depth filters. Barcoding would have been averted through genetic, rather than optical map-based physical ordering of SNPs. By contrast, our study benefitted from both the new physical map and from utilization of a data set that required little filtering and was not subjected to imputation.

Prospects and contingencies

Based on the data presented in this study, the evidence for the widespread presence of non-parental haplotypes in the IBMsyn10DHL resource is unequivocal. It is less clear exactly how it should be treated. In our view, the new masked map is the best map produced to date because it is not tainted by as many non-parental segments. However, more work will be needed in order to empirically determine the potential gains in precision and accuracy that we forecast.

Based on the absence of masking in the Liu et al. (2015a) data set, as well as on the absence of barcoding, we conclude that the local order of many SNPs in the map must be incorrect. We also suggest that the observed high levels of segregation distortion and map expansion they report are to some degree artefactual, although a more careful treatment of these claims must be undertaken in order to determine whether or not a different map construction approach would lead to a map that performs better for bi-parental QTL mapping.

Thus far, no study has carefully examined the relative uniqueness and physical distribution of cross-over events across the DHLs, nor has the level of genetic detail required to unveil these recombinations been discerned. Both of these efforts will be required in order to understand the nature of the gains in genetic resolution that this resource may afford to experimentalists. Moreover, we must collectively utilize these data to determine the utility of the breeding approach that was employed. There is no clear record of the effective population size maintained during intermating, nor of the individual plant utilization schemes. The numbers of syn10 families that contributed to the DH induction crosses is also missing from the record. These points are not intended as criticisms; it is important to remember that synthetic intermating may have initially been undertaken as a breeding exercise, rather than as the foundation for a high resolution genetic resource to be dissected some 30 years later. Either way, there should be additional discussions concerning how intermating and doubled haploidization should be conducted in order to maximize achievable genetic resolution on a per line basis. The new and improved genetic map for the IBMSyn10DHL population and its integration with the B73 RefGen_v4 physical map are expected to provide a new starting point for these discussions.

Figures and Tables

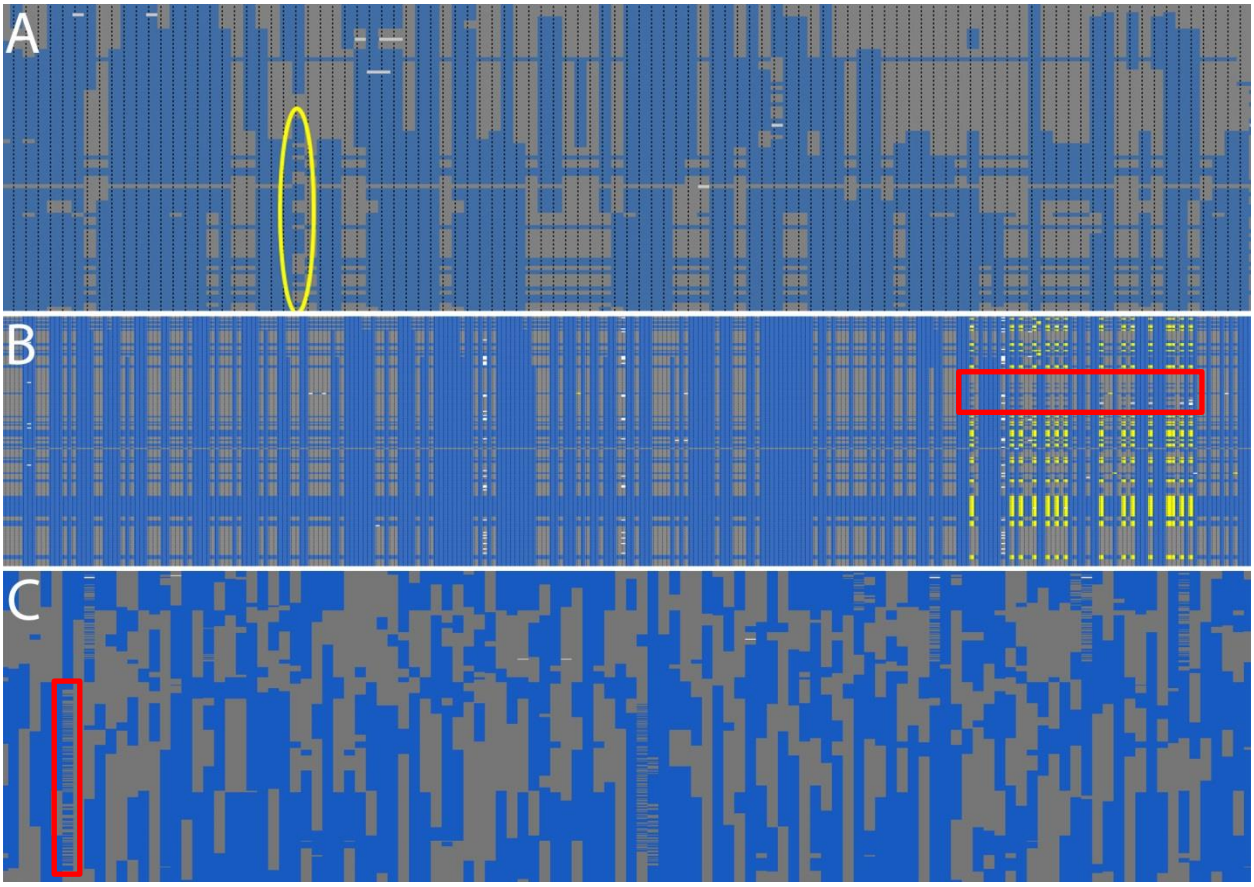

Figure 1: Graphical representations displaying detection of artificial polymorphisms and improvements derived from their removal. **A.** In this view, a series of horizontal stripes arising from nearly monomorphic markers is primarily due to a single DHL harboring SNPs at sites that do not differ between B73 and Mo17 (yellow oval). **B.** A genomic region showing conservation of artificial polymorphisms (yellow highlights) across several DHLs, demonstrating a case where nearly 10% of the lines are affected for this genetic region. Note that in the contaminated DHLs, "barcodes" are evident, indicating improbably high rates of recombination among SNPs that are actually polymorphic between B73 and Mo17 (Red box, SNP data not highlighted in yellow). These barcodes are a signature of contamination, but alone, should not be used for data masking, which is best guided by the presence of artificial polymorphisms. **C.** After removal of artificially polymorphic markers, horizontal striping disappears, revealing "barcodes" (red box, one example), the secondary graphical signature of non-parental haplotype contamination.

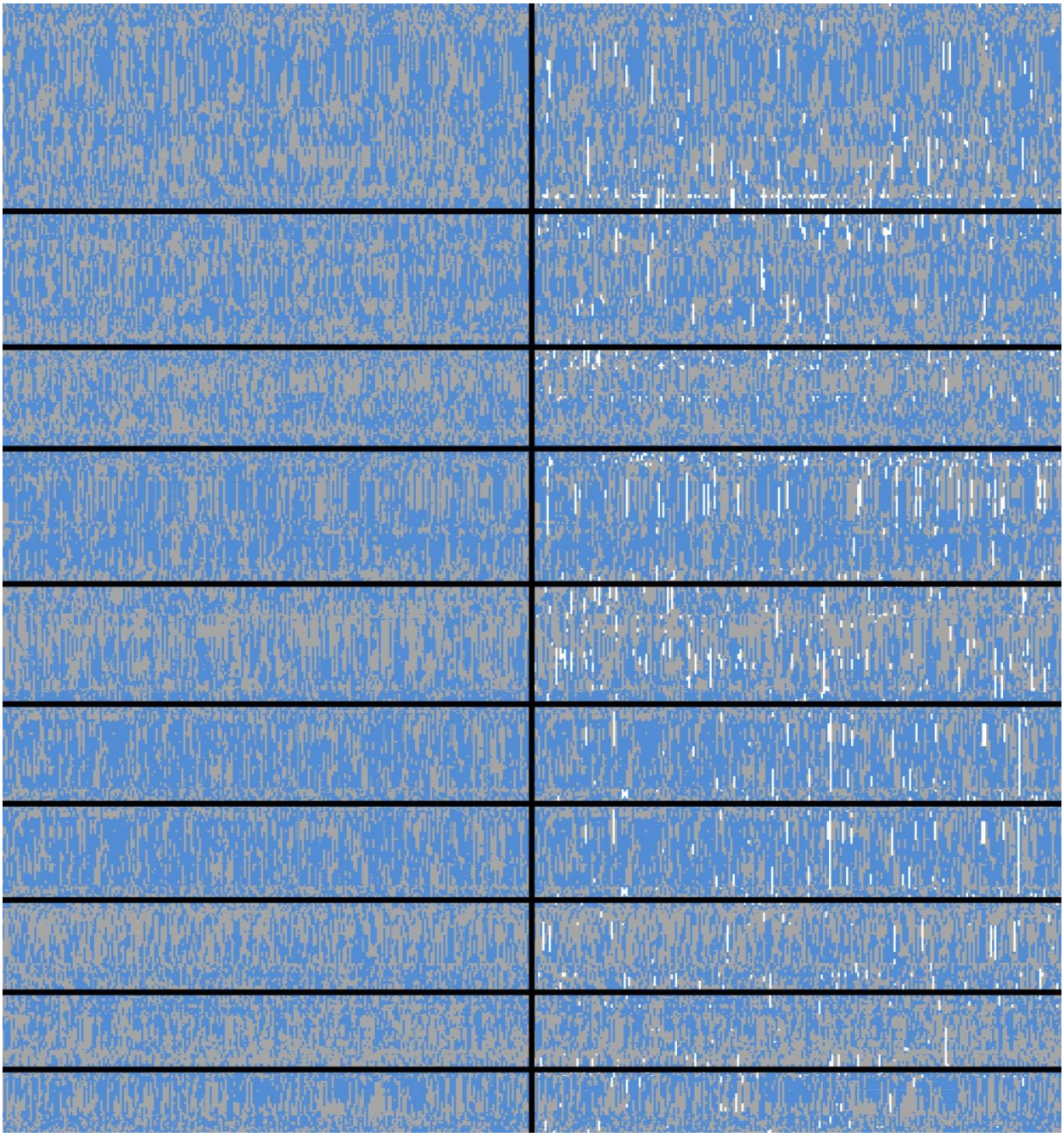

Figure 2: Graphical displays of genetic recombination and missing data for the entire unmasked (left side) and masked (right side) versions of the 10 maize chromosomes (top to bottom) as captured using the 6,745 SNP by 247 DHL genotype data set. Masked regions are shown in white and represent 3.17% of the data in this matrix, although only 2.80% of the missing data in the finalized genetic map built from the masked data set.

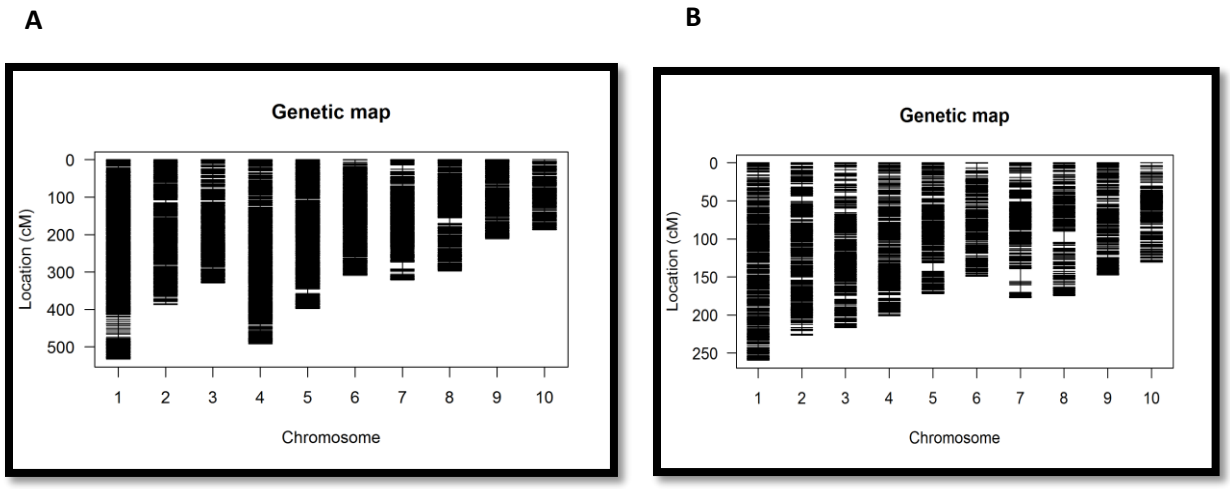

Figure 3: Comparison of genetic maps for the unmasked and masked versions of the IBMsyn10DHL resource as constructed from 247 DHLs. Each SNP is depicted as a horizontal black line, such that degree of blackness indicates higher density of genetic markers. **A.** The unmasked map contains 6,745 markers and is 3,465cM in length. **B.** The masked map contains 4,191 markers and is 1,854cM in length. Note the scale differences between the two maps, as well as the correspondence among positions with the largest intermarker distances (white gaps).

Table 1: List of 414 SX19syn10DHL Identifiers with inclusion status and justification

IBMsyn10DHL Identifier	Seed stock and SNP Availability	Exclusion Criterion
SX19S10_M0001	Union set of 332	none, present in set of 247
SX19S10_M0002	Union set of 332	none, present in set of 247
SX19S10_M0006	Union set of 332	none, present in set of 247
SX19S10_M0008	Union set of 332	none, present in set of 247
SX19S10_M0009	Union set of 332	none, present in set of 247
SX19S10_M0010	Union set of 332	none, present in set of 247
SX19S10_M0011	Union set of 332	none, present in set of 247
SX19S10_M0017	Union set of 332	none, present in set of 247
SX19S10_M0018	Union set of 332	none, present in set of 247
SX19S10_M0019	Union set of 332	none, present in set of 247
SX19S10_M0020	Union set of 332	none, present in set of 247
SX19S10_M0024	Union set of 332	none, present in set of 247
SX19S10_M0027	Union set of 332	none, present in set of 247
SX19S10_M0029	Union set of 332	none, present in set of 247
SX19S10_M0030	Union set of 332	none, present in set of 247
SX19S10_M0032	Union set of 332	none, present in set of 247
SX19S10_M0034	Union set of 332	none, present in set of 247
SX19S10_M0036	Union set of 332	none, present in set of 247
SX19S10_M0038	Union set of 332	none, present in set of 247
SX19S10_M0041	Union set of 332	none, present in set of 247

Table 1: Continued

SX19S10_M0042	Union set of 332	none, present in set of 247
SX19S10_M0047	Union set of 332	none, present in set of 247
SX19S10_M0049	Union set of 332	none, present in set of 247
SX19S10_M0050	Union set of 332	none, present in set of 247
SX19S10_M0053	Union set of 332	none, present in set of 247
SX19S10_M0056	Union set of 332	none, present in set of 247
SX19S10_M0057	Union set of 332	none, present in set of 247
SX19S10_M0059	Union set of 332	none, present in set of 247
SX19S10_M0061	Union set of 332	none, present in set of 247
SX19S10_M0062	Union set of 332	none, present in set of 247
SX19S10_M0064	Union set of 332	none, present in set of 247
SX19S10_M0070	Union set of 332	none, present in set of 247
SX19S10_M0072	Union set of 332	none, present in set of 247
SX19S10_M0074	Union set of 332	none, present in set of 247
SX19S10_M0076	Union set of 332	none, present in set of 247
SX19S10_M0078	Union set of 332	none, present in set of 247
SX19S10_M0082	Union set of 332	none, present in set of 247
SX19S10_M0083	Union set of 332	none, present in set of 247
SX19S10_M0084	Union set of 332	none, present in set of 247
SX19S10_M0086	Union set of 332	none, present in set of 247
SX19S10_M0101	Union set of 332	none, present in set of 247
SX19S10_M0105	Union set of 332	none, present in set of 247
SX19S10_M0109	Union set of 332	none, present in set of 247
SX19S10_M0111	Union set of 332	none, present in set of 247
SX19S10_M0112	Union set of 332	none, present in set of 247
SX19S10_M0116	Union set of 332	none, present in set of 247
SX19S10_M0118	Union set of 332	none, present in set of 247
SX19S10_M0119	Union set of 332	none, present in set of 247
SX19S10_M0120	Union set of 332	none, present in set of 247
SX19S10_M0129	Union set of 332	none, present in set of 247
SX19S10_M0130	Union set of 332	none, present in set of 247
SX19S10_M0136	Union set of 332	none, present in set of 247
SX19S10_M0138	Union set of 332	none, present in set of 247
SX19S10_M0145	Union set of 332	none, present in set of 247
SX19S10_M0146	Union set of 332	none, present in set of 247
SX19S10_M0152	Union set of 332	none, present in set of 247
SX19S10_M0156	Union set of 332	none, present in set of 247
SX19S10_M0158	Union set of 332	none, present in set of 247

Table 1: Continued

SX19S10_M0159	Union set of 332	none, present in set of 247
SX19S10_M0166	Union set of 332	none, present in set of 247
SX19S10_M0169	Union set of 332	none, present in set of 247
SX19S10_M0171	Union set of 332	none, present in set of 247
SX19S10_M0175	Union set of 332	none, present in set of 247
SX19S10_M0177	Union set of 332	none, present in set of 247
SX19S10_M0179	Union set of 332	none, present in set of 247
SX19S10_M0184	Union set of 332	none, present in set of 247
SX19S10_M0188	Union set of 332	none, present in set of 247
SX19S10_M0189	Union set of 332	none, present in set of 247
SX19S10_M0195	Union set of 332	none, present in set of 247
SX19S10_M0196	Union set of 332	none, present in set of 247
SX19S10_M0198	Union set of 332	none, present in set of 247
SX19S10_M0202	Union set of 332	none, present in set of 247
SX19S10_M0203	Union set of 332	none, present in set of 247
SX19S10_M0206	Union set of 332	none, present in set of 247
SX19S10_M0208	Union set of 332	none, present in set of 247
SX19S10_M0209	Union set of 332	none, present in set of 247
SX19S10_M0210	Union set of 332	none, present in set of 247
SX19S10_M0212	Union set of 332	none, present in set of 247
SX19S10_M0215	Union set of 332	none, present in set of 247
SX19S10_M0217	Union set of 332	none, present in set of 247
SX19S10_M0218	Union set of 332	none, present in set of 247
SX19S10_M0221	Union set of 332	none, present in set of 247
SX19S10_M0227	Union set of 332	none, present in set of 247
SX19S10_M0230	Union set of 332	none, present in set of 247
SX19S10_M0231	Union set of 332	none, present in set of 247
SX19S10_M0234	Union set of 332	none, present in set of 247
SX19S10_M0237	Union set of 332	none, present in set of 247
SX19S10_M0239	Union set of 332	none, present in set of 247
SX19S10_M0243	Union set of 332	none, present in set of 247
SX19S10_M0244	Union set of 332	none, present in set of 247
SX19S10_M0249	Union set of 332	none, present in set of 247
SX19S10_M0250	Union set of 332	none, present in set of 247
SX19S10_M0252	Union set of 332	none, present in set of 247
SX19S10_M0253	Union set of 332	none, present in set of 247
SX19S10_M0254	Union set of 332	none, present in set of 247
SX19S10_M0256	Union set of 332	none, present in set of 247

Table 1: Continued

SX19S10_M0258	Union set of 332	none, present in set of 247
SX19S10_M0260	Union set of 332	none, present in set of 247
SX19S10_M0261	Union set of 332	none, present in set of 247
SX19S10_M0262	Union set of 332	none, present in set of 247
SX19S10_M0264	Union set of 332	none, present in set of 247
SX19S10_M0267	Union set of 332	none, present in set of 247
SX19S10_M0272	Union set of 332	none, present in set of 247
SX19S10_M0286	Union set of 332	none, present in set of 247
SX19S10_M0289	Union set of 332	none, present in set of 247
SX19S10_M0290	Union set of 332	none, present in set of 247
SX19S10_M0295	Union set of 332	none, present in set of 247
SX19S10_M0298	Union set of 332	none, present in set of 247
SX19S10_M0304	Union set of 332	none, present in set of 247
SX19S10_M0310	Union set of 332	none, present in set of 247
SX19S10_M0312	Union set of 332	none, present in set of 247
SX19S10_M0316	Union set of 332	none, present in set of 247
SX19S10_M0323	Union set of 332	none, present in set of 247
SX19S10_M0324	Union set of 332	none, present in set of 247
SX19S10_M0328	Union set of 332	none, present in set of 247
SX19S10_M0330	Union set of 332	none, present in set of 247
SX19S10_M0332	Union set of 332	none, present in set of 247
SX19S10_M0334	Union set of 332	none, present in set of 247
SX19S10_M0335	Union set of 332	none, present in set of 247
SX19S10_M0336	Union set of 332	none, present in set of 247
SX19S10_M0340	Union set of 332	none, present in set of 247
SX19S10_M0345	Union set of 332	none, present in set of 247
SX19S10_M0346	Union set of 332	none, present in set of 247
SX19S10_M0347	Union set of 332	none, present in set of 247
SX19S10_M0349	Union set of 332	none, present in set of 247
SX19S10_M0356	Union set of 332	none, present in set of 247
SX19S10_M0357	Union set of 332	none, present in set of 247
SX19S10_M0358	Union set of 332	none, present in set of 247
SX19S10_M0372	Union set of 332	none, present in set of 247
SX19S10_M0375	Union set of 332	none, present in set of 247
SX19S10_M0376	Union set of 332	none, present in set of 247
SX19S10_M0379	Union set of 332	none, present in set of 247
SX19S10_M0381	Union set of 332	none, present in set of 247
SX19S10_M0383	Union set of 332	none, present in set of 247
SX19S10_M0384	Union set of 332	none, present in set of 247
SX19S10_M0388	Union set of 332	none, present in set of 247

Table 1: Continued

SX19S10_M0389	Union set of 332	none, present in set of 247
SX19S10_M0390	Union set of 332	none, present in set of 247
SX19S10_M0391	Union set of 332	none, present in set of 247
SX19S10_M0396	Union set of 332	none, present in set of 247
SX19S10_M0404	Union set of 332	none, present in set of 247
SX19S10_M0405	Union set of 332	none, present in set of 247
SX19S10_M0407	Union set of 332	none, present in set of 247
SX19S10_M0412	Union set of 332	none, present in set of 247
SX19S10_M0422	Union set of 332	none, present in set of 247
SX19S10_M0424	Union set of 332	none, present in set of 247
SX19S10_M0428	Union set of 332	none, present in set of 247
SX19S10_M0429	Union set of 332	none, present in set of 247
SX19S10_M0430	Union set of 332	none, present in set of 247
SX19S10_M0433	Union set of 332	none, present in set of 247
SX19S10_M0434	Union set of 332	none, present in set of 247
SX19S10_M0436	Union set of 332	none, present in set of 247
SX19S10_M0438	Union set of 332	none, present in set of 247
SX19S10_M0442	Union set of 332	none, present in set of 247
SX19S10_M0443	Union set of 332	none, present in set of 247
SX19S10_M0444	Union set of 332	none, present in set of 247
SX19S10_M0446	Union set of 332	none, present in set of 247
SX19S10_M0447	Union set of 332	none, present in set of 247
SX19S10_M0448	Union set of 332	none, present in set of 247
SX19S10_M0449	Union set of 332	none, present in set of 247
SX19S10_M0450	Union set of 332	none, present in set of 247
SX19S10_M0452	Union set of 332	none, present in set of 247
SX19S10_M0453	Union set of 332	none, present in set of 247
SX19S10_M0454	Union set of 332	none, present in set of 247
SX19S10_M0473	Union set of 332	none, present in set of 247
SX19S10_M0476	Union set of 332	none, present in set of 247
SX19S10_M0481	Union set of 332	none, present in set of 247
SX19S10_M0487	Union set of 332	none, present in set of 247
SX19S10_M0489	Union set of 332	none, present in set of 247
SX19S10_M0490	Union set of 332	none, present in set of 247
SX19S10_M0491	Union set of 332	none, present in set of 247
SX19S10_M0494	Union set of 332	none, present in set of 247
SX19S10_M0498	Union set of 332	none, present in set of 247
SX19S10_M0499	Union set of 332	none, present in set of 247
SX19S10_M0500	Union set of 332	none, present in set of 247
SX19S10_M0501	Union set of 332	none, present in set of 247

Table 1: Continued

SX19S10_M0502	Union set of 332	none, present in set of 247
SX19S10_M0504	Union set of 332	none, present in set of 247
SX19S10_M0506	Union set of 332	none, present in set of 247
SX19S10_M0507	Union set of 332	none, present in set of 247
SX19S10_M0509	Union set of 332	none, present in set of 247
SX19S10_M0510	Union set of 332	none, present in set of 247
SX19S10_M0512	Union set of 332	none, present in set of 247
SX19S10_M0515	Union set of 332	none, present in set of 247
SX19S10_M0517	Union set of 332	none, present in set of 247
SX19S10_M0519	Union set of 332	none, present in set of 247
SX19S10_M0525	Union set of 332	none, present in set of 247
SX19S10_M0527	Union set of 332	none, present in set of 247
SX19S10_M0533	Union set of 332	none, present in set of 247
SX19S10_M0535	Union set of 332	none, present in set of 247
SX19S10_M0537	Union set of 332	none, present in set of 247
SX19S10_M0538	Union set of 332	none, present in set of 247
SX19S10_M0541	Union set of 332	none, present in set of 247
SX19S10_M0543	Union set of 332	none, present in set of 247
SX19S10_M0544	Union set of 332	none, present in set of 247
SX19S10_M0545	Union set of 332	none, present in set of 247
SX19S10_M0546	Union set of 332	none, present in set of 247
SX19S10_M0547	Union set of 332	none, present in set of 247
SX19S10_M0548	Union set of 332	none, present in set of 247
SX19S10_M0549	Union set of 332	none, present in set of 247
SX19S10_M0552	Union set of 332	none, present in set of 247
SX19S10_M0553	Union set of 332	none, present in set of 247
SX19S10_M0556	Union set of 332	none, present in set of 247
SX19S10_M0557	Union set of 332	none, present in set of 247
SX19S10_M0559	Union set of 332	none, present in set of 247
SX19S10_M0560	Union set of 332	none, present in set of 247
SX19S10_M0561	Union set of 332	none, present in set of 247
SX19S10_M0562	Union set of 332	none, present in set of 247
SX19S10_M0563	Union set of 332	none, present in set of 247
SX19S10_M0564	Union set of 332	none, present in set of 247
SX19S10_M0565	Union set of 332	none, present in set of 247
SX19S10_M0566	Union set of 332	none, present in set of 247
SX19S10_M0567	Union set of 332	none, present in set of 247
SX19S10_M0569	Union set of 332	none, present in set of 247
SX19S10_M0571	Union set of 332	none, present in set of 247
SX19S10_M0572	Union set of 332	none, present in set of 247

Table 1: Continued

SX19S10_M0573	Union set of 332	none, present in set of 247
SX19S10_M0574	Union set of 332	none, present in set of 247
SX19S10_M0575	Union set of 332	none, present in set of 247
SX19S10_M0577	Union set of 332	none, present in set of 247
SX19S10_M0580	Union set of 332	none, present in set of 247
SX19S10_M0581	Union set of 332	none, present in set of 247
SX19S10_M0583	Union set of 332	none, present in set of 247
SX19S10_M0585	Union set of 332	none, present in set of 247
SX19S10_M0587	Union set of 332	none, present in set of 247
SX19S10_M0588	Union set of 332	none, present in set of 247
SX19S10_M0589	Union set of 332	none, present in set of 247
SX19S10_M0590	Union set of 332	none, present in set of 247
SX19S10_M0592	Union set of 332	none, present in set of 247
SX19S10_M0594	Union set of 332	none, present in set of 247
SX19S10_M0598	Union set of 332	none, present in set of 247
SX19S10_M0602	Union set of 332	none, present in set of 247
SX19S10_M0607	Union set of 332	none, present in set of 247
SX19S10_M0609	Union set of 332	none, present in set of 247
SX19S10_M0610	Union set of 332	none, present in set of 247
SX19S10_M0611	Union set of 332	none, present in set of 247
SX19S10_M0614	Union set of 332	none, present in set of 247
SX19S10_M0618	Union set of 332	none, present in set of 247
SX19S10_M0619	Union set of 332	none, present in set of 247
SX19S10_M0623	Union set of 332	none, present in set of 247
SX19S10_M0624	Union set of 332	none, present in set of 247
SX19S10_M0630	Union set of 332	none, present in set of 247
SX19S10_M0634	Union set of 332	none, present in set of 247
SX19S10_M0639	Union set of 332	none, present in set of 247
SX19S10_M0641	Union set of 332	none, present in set of 247
SX19S10_M0642	Union set of 332	none, present in set of 247
SX19S10_M0645	Union set of 332	none, present in set of 247
SX19S10_M0004	Union set of 332	>10% initial missing data
SX19S10_M0015	Union set of 332	>10% initial missing data
SX19S10_M0043	Union set of 332	>10% initial missing data
SX19S10_M0051	Union set of 332	>10% initial missing data
SX19S10_M0054	Union set of 332	>10% initial missing data
SX19S10_M0066	Union set of 332	>10% initial missing data
SX19S10_M0068	Union set of 332	>10% initial missing data
SX19S10_M0091	Union set of 332	>10% initial missing data
SX19S10_M0095	Union set of 332	>10% initial missing data

Table 1: Continued

SX19S10_M0108	Union set of 332	>10% initial missing data
SX19S10_M0110	Union set of 332	>10% initial missing data
SX19S10_M0126	Union set of 332	>10% initial missing data
SX19S10_M0164	Union set of 332	>10% initial missing data
SX19S10_M0178	Union set of 332	>10% initial missing data
SX19S10_M0186	Union set of 332	>10% initial missing data
SX19S10_M0193	Union set of 332	>10% initial missing data
SX19S10_M0214	Union set of 332	>10% initial missing data
SX19S10_M0240	Union set of 332	>10% initial missing data
SX19S10_M0242	Union set of 332	>10% initial missing data
SX19S10_M0266	Union set of 332	>10% initial missing data
SX19S10_M0285	Union set of 332	>10% initial missing data
SX19S10_M0293	Union set of 332	>10% initial missing data
SX19S10_M0296	Union set of 332	>10% initial missing data
SX19S10_M0309	Union set of 332	>10% initial missing data
SX19S10_M0314	Union set of 332	>10% initial missing data
SX19S10_M0315	Union set of 332	>10% initial missing data
SX19S10_M0341	Union set of 332	>10% initial missing data
SX19S10_M0373	Union set of 332	>10% initial missing data
SX19S10_M0374	Union set of 332	>10% initial missing data
SX19S10_M0377	Union set of 332	>10% initial missing data
SX19S10_M0382	Union set of 332	>10% initial missing data
SX19S10_M0414	Union set of 332	>10% initial missing data
SX19S10_M0415	Union set of 332	>10% initial missing data
SX19S10_M0427	Union set of 332	>10% initial missing data
SX19S10_M0471	Union set of 332	>10% initial missing data
SX19S10_M0472	Union set of 332	>10% initial missing data
SX19S10_M0474	Union set of 332	>10% initial missing data
SX19S10_M0492	Union set of 332	>10% initial missing data
SX19S10_M0493	Union set of 332	>10% initial missing data
SX19S10_M0505	Union set of 332	>10% initial missing data
SX19S10_M0536	Union set of 332	>10% initial missing data
SX19S10_M0550	Union set of 332	>10% initial missing data
SX19S10_M0597	Union set of 332	>10% initial missing data
SX19S10_M0613	Union set of 332	>10% initial missing data
SX19S10_M0617	Union set of 332	>10% initial missing data
SX19S10_M0625	Union set of 332	>10% initial missing data
SX19S10_M0627	Union set of 332	>10% initial missing data
SX19S10_M0632	Union set of 332	>10% initial missing data
SX19S10_M0636	Union set of 332	>10% initial missing data

Table 1: Continued

SX19S10_M0023	Union set of 332	>2% non-parental alleles
SX19S10_M0025	Union set of 332	>2% non-parental alleles
SX19S10_M0093	Union set of 332	>2% non-parental alleles
SX19S10_M0114	Union set of 332	>2% non-parental alleles
SX19S10_M0124	Union set of 332	>2% non-parental alleles
SX19S10_M0155	Union set of 332	>2% non-parental alleles
SX19S10_M0162	Union set of 332	>2% non-parental alleles
SX19S10_M0168	Union set of 332	>2% non-parental alleles
SX19S10_M0173	Union set of 332	>2% non-parental alleles
SX19S10_M0220	Union set of 332	>2% non-parental alleles
SX19S10_M0223	Union set of 332	>2% non-parental alleles
SX19S10_M0245	Union set of 332	>2% non-parental alleles
SX19S10_M0273	Union set of 332	>2% non-parental alleles
SX19S10_M0287	Union set of 332	>2% non-parental alleles
SX19S10_M0303	Union set of 332	>2% non-parental alleles
SX19S10_M0305	Union set of 332	>2% non-parental alleles
SX19S10_M0308	Union set of 332	>2% non-parental alleles
SX19S10_M0319	Union set of 332	>2% non-parental alleles
SX19S10_M0320	Union set of 332	>2% non-parental alleles
SX19S10_M0343	Union set of 332	>2% non-parental alleles
SX19S10_M0385	Union set of 332	>2% non-parental alleles
SX19S10_M0431	Union set of 332	>2% non-parental alleles
SX19S10_M0457	Union set of 332	>2% non-parental alleles
SX19S10_M0461	Union set of 332	>2% non-parental alleles
SX19S10_M0463	Union set of 332	>2% non-parental alleles
SX19S10_M0465	Union set of 332	>2% non-parental alleles
SX19S10_M0466	Union set of 332	>2% non-parental alleles
SX19S10_M0469	Union set of 332	>2% non-parental alleles
SX19S10_M0483	Union set of 332	>2% non-parental alleles
SX19S10_M0484	Union set of 332	>2% non-parental alleles
SX19S10_M0540	Union set of 332	>2% non-parental alleles
SX19S10_M0570	Union set of 332	>2% non-parental alleles
SX19S10_M0586	Union set of 332	>2% non-parental alleles
SX19S10_M0606	Union set of 332	>2% non-parental alleles
SX19S10_M0638	Union set of 332	>2% non-parental alleles
SX19S10_M0495	Union set of 332	Seed propagation failure
SX19S10_M0012	SNP data only	SNP data only
SX19S10_M0055	SNP data only	SNP data only
SX19S10_M0063	SNP data only	SNP data only
SX19S10_M0069	SNP data only	SNP data only

Table 1: Continued

SX19S10_M0079	SNP data only	SNP data only
SX19S10_M0090	SNP data only	SNP data only
SX19S10_M0092	SNP data only	SNP data only
SX19S10_M0103	SNP data only	SNP data only
SX19S10_M0117	SNP data only	SNP data only
SX19S10_M0172	SNP data only	SNP data only
SX19S10_M0311	SNP data only	SNP data only
SX19S10_M0317	SNP data only	SNP data only
SX19S10_M0366	SNP data only	SNP data only
SX19S10_M0401	SNP data only	SNP data only
SX19S10_M0477	SNP data only	SNP data only
SX19S10_M0479	SNP data only	SNP data only
SX19S10_M0513	SNP data only	SNP data only
SX19S10_M0532	SNP data only	SNP data only
SX19S10_M0534	SNP data only	SNP data only
SX19S10_M0542	SNP data only	SNP data only
SX19S10_M0551	SNP data only	SNP data only
SX19S10_M0558	SNP data only	SNP data only
SX19S10_M0568	SNP data only	SNP data only
SX19S10_M0647	SNP data only	SNP data only
SX19S10_M0007	Seed accessions only	Seed accessions only
SX19S10_M0065	Seed accessions only	Seed accessions only
SX19S10_M0080	Seed accessions only	Seed accessions only
SX19S10_M0081	Seed accessions only	Seed accessions only
SX19S10_M0131	Seed accessions only	Seed accessions only
SX19S10_M0132	Seed accessions only	Seed accessions only
SX19S10_M0144	Seed accessions only	Seed accessions only
SX19S10_M0161	Seed accessions only	Seed accessions only
SX19S10_M0170	Seed accessions only	Seed accessions only
SX19S10_M0176	Seed accessions only	Seed accessions only
SX19S10_M0187	Seed accessions only	Seed accessions only
SX19S10_M0190	Seed accessions only	Seed accessions only
SX19S10_M0194	Seed accessions only	Seed accessions only
SX19S10_M0229	Seed accessions only	Seed accessions only
SX19S10_M0236	Seed accessions only	Seed accessions only
SX19S10_M0248	Seed accessions only	Seed accessions only
SX19S10_M0251	Seed accessions only	Seed accessions only
SX19S10_M0268	Seed accessions only	Seed accessions only
SX19S10_M0275	Seed accessions only	Seed accessions only
SX19S10_M0292	Seed accessions only	Seed accessions only

Table 1: Continued

SX19S10_M0294	Seed accessions only	Seed accessions only
SX19S10_M0327	Seed accessions only	Seed accessions only
SX19S10_M0329	Seed accessions only	Seed accessions only
SX19S10_M0337	Seed accessions only	Seed accessions only
SX19S10_M0338	Seed accessions only	Seed accessions only
SX19S10_M0342	Seed accessions only	Seed accessions only
SX19S10_M0344	Seed accessions only	Seed accessions only
SX19S10_M0350	Seed accessions only	Seed accessions only
SX19S10_M0351	Seed accessions only	Seed accessions only
SX19S10_M0362	Seed accessions only	Seed accessions only
SX19S10_M0368	Seed accessions only	Seed accessions only
SX19S10_M0371	Seed accessions only	Seed accessions only
SX19S10_M0378	Seed accessions only	Seed accessions only
SX19S10_M0380	Seed accessions only	Seed accessions only
SX19S10_M0394	Seed accessions only	Seed accessions only
SX19S10_M0395	Seed accessions only	Seed accessions only
SX19S10_M0402	Seed accessions only	Seed accessions only
SX19S10_M0409	Seed accessions only	Seed accessions only
SX19S10_M0419	Seed accessions only	Seed accessions only
SX19S10_M0421	Seed accessions only	Seed accessions only
SX19S10_M0425	Seed accessions only	Seed accessions only
SX19S10_M0426	Seed accessions only	Seed accessions only
SX19S10_M0437	Seed accessions only	Seed accessions only
SX19S10_M0440	Seed accessions only	Seed accessions only
SX19S10_M0441	Seed accessions only	Seed accessions only
SX19S10_M0445	Seed accessions only	Seed accessions only
SX19S10_M0451	Seed accessions only	Seed accessions only
SX19S10_M0455	Seed accessions only	Seed accessions only
SX19S10_M0458	Seed accessions only	Seed accessions only
SX19S10_M0459	Seed accessions only	Seed accessions only
SX19S10_M0460	Seed accessions only	Seed accessions only
SX19S10_M0464	Seed accessions only	Seed accessions only
SX19S10_M0470	Seed accessions only	Seed accessions only
SX19S10_M0475	Seed accessions only	Seed accessions only
SX19S10_M0478	Seed accessions only	Seed accessions only
SX19S10_M0480	Seed accessions only	Seed accessions only
SX19S10_M0539	Seed accessions only	Seed accessions only
SX19S10_M0616	Seed accessions only	Seed accessions only

Table 2: iPLEX Gold assay design for 36 quality control SNPs utilized to confirm line identities.

Chromosome	SNP ID	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
Ch1	PZE-101104306	ACGTTGGATGTAGCCATCTATTGCAGCAGG	ACGTTGGATGGTCTCGGATACGCACTTCTG	ggggcGGCACGTCGCTGTTG
Ch1	SYN25705	ACGTTGGATGAGGAGCTTTTCAAGCACCTC	ACGTTGGATGTTCAACGAAGGCAACACGAG	CACGTGATCTTCCAGCAA
Ch1	PZE-101110644	ACGTTGGATGCTAAAGGTTTGTAGACCGGC	ACGTTGGATGAGTAGCTTGGCCTCCTTTTG	gAATCTTTATTTGAACAAACGGG
Ch1	SYN36726	ACGTTGGATGCTTGGACTTACCTGATGGAG	ACGTTGGATGCAGTGAATCAACAGTTGAC	TTGCTGATTGTGTGGC
Ch3	SYN25147	ACGTTGGATGGAAGTTTTCGTGCTCGGAAC	ACGTTGGATGGCAGACAATGTCCAAGAACC	cccccATACACGTCATCGCCTG
Ch3	SYN32032	ACGTTGGATGGCGCGTGGGTTGTGGCTGT	ACGTTGGATGAAGACCAAACAGAGAGGGAG	CACAATACCGAACCGAAATAC
Ch3	SYN10296	ACGTTGGATGTTCGGTTCGAAGATCAGAGG	ACGTTGGATGGCCTTTGATTCTGTTGTAGAG	tTAGTATATGACTGGCGCA
Ch3	SYN8370	ACGTTGGATGAGGTATGGATCATGGGATGG	ACGTTGGATGTGGCGGTCTTGTATAGGAAC	CCCATAGCGATGACC
Ch4	PZE-104091158	ACGTTGGATGAGCGGTTCGGAGATGAAATC	ACGTTGGATGGACGAATTGACGAGCAACTG	ATCCGCGGTTTTGTGACTA
Ch4	SYN8267	ACGTTGGATGGCCCAATGCATCTGAAAATG	ACGTTGGATGAGGGCTCAAGCGATAACTTC	cacgcTGTCATCATCTTGAATACCA
Ch4	SYN2206	ACGTTGGATGCCCAGTTCTTTTTCCCACAC	ACGTTGGATGTTCTGGTAGCGACGACTTTG	cAGCACTGTTCCCTGTATAAAAAAA
Ch4	PZE-104094133	ACGTTGGATGTCGCCTCGTGATTGGAGTG	ACGTTGGATGGGCGAAAAAAGAGGACCAAG	aTGCCTTCCCTTCCCT
Ch6	SYN36205	ACGTTGGATGCCAAAGTCCTAGTGTTACCG	ACGTTGGATGTAACCCAAGAAACGGTGCTC	ggggaATTTTCATGTTTGTGTAACAGTT
Ch6	SYN8312	ACGTTGGATGACTGGTACCGCAATGATCAC	ACGTTGGATGTGGATTACGACTTTGGTGGC	ttcttGTACCGCAATGATCACACACTAG
Ch7	SYN15108	ACGTTGGATGGTATGCAGTGAATTGGGCTC	ACGTTGGATGCCTTTTGCCGGACATGAATG	gGGTACCAGACAGAGGA
Ch7	SYN31213	ACGTTGGATGATCGGACTCTCCTCTTCTTG	ACGTTGGATGTGTACCCACAGGTCAGCGG	accccTGGGCGGGGTGGAATGGGGTG
Ch7	SYN12273	ACGTTGGATGATGATCGGCCAGACTTCTTG	ACGTTGGATGATTCAACGCAATGGTGTCCG	gggaGTCCGGGCTGTTCAAACT
Ch7	SYN13508	ACGTTGGATGACGACGGCGAGGACGAGCA	ACGTTGGATGATTCTTCTCGCCTTACTCCC	aCTCACAGAACCCAGTAAAATAT
Ch8	SYN19540	ACGTTGGATGAGCTGTGGCTGCATCAGTTG	ACGTTGGATGATGTCGCATCCCATTGTCAC	aaaggCCCATTGTCACTCGATTGTTAAG
Ch8	SYN21760	ACGTTGGATGTACGGATTCGGATAGATCGG	ACGTTGGATGATCCGTCTAAGAGGACTGAG	gaggCGGATAGATCGGATACTGAATAC
Ch8	PZE-108042778	ACGTTGGATGGCATGCAAATATATGATCGTC	ACGTTGGATGAGCAAGCAACATAATCTCAC	ggtggATGATCGTCATGAAGGCTGC
Ch8	SYN3784	ACGTTGGATGCAGGAACCAAGCAAGTTGAC	ACGTTGGATGCTTGGGAAGGAAACAGTCAC	aagtAAACAGTCACTAACCCTGGGGCTT
Ch9	SYN33496	ACGTTGGATGGGCCAAGGCGCAGGTGGAG	ACGTTGGATGGATGGGTCATCATCCATCTG	ccccTGGCCTCCGCCTCGT
Ch9	SYN34041	ACGTTGGATGTTGCAGAGGAGCAATGATAG	ACGTTGGATGGTATTGTTCAGCCTCTTCAG	GGAGCAATGATAGATCAAGG
Ch9	SYN36472	ACGTTGGATGACATAGAAAGAGAGAGGCCG	ACGTTGGATGACGACGCCGACCCTACTGC	agtCCGGGATCGATATCGCGATAG
Ch9	SYN37647	ACGTTGGATGGGTACGTCGAGAATGTCATC	ACGTTGGATGAAGCAGCAACGGAGAGCAAG	ccCTGGCATGTCACGGC

Table 2: Continued

Ch9	SYN8354	ACGTTGGATGTGGAGGTGAGAGTTCAAACG	ACGTTGGATGCTGTGCCATTGTTTGGCATC	ttcacTTGTTTGGCATCTCCATA
Ch9	SYN8851	ACGTTGGATGTAAATCAGGAGGGTGCTGTG	ACGTTGGATGCATAAAGTCCTGAAGCACCG	CGTGATAGACCGCTC
Ch9	SYN24333	ACGTTGGATGTCGGACACAAGAACCTCTTC	ACGTTGGATGCAAGGCTTACACACGTAAGG	cctttTTACACACGTAAGGGCACAGA
Ch9	SYN6090	ACGTTGGATGCTGGAAGATGGTCAAAGACG	ACGTTGGATGGGAGTCCAGCTTCAAGAATG	cGCTTCAAGAATGAATAACTTCAGAA
Ch10	SYN15277	ACGTTGGATGGGGAGTTCTTCAAATGTCTC	ACGTTGGATGTTGTTCTTGAGTTGGAGCAC	tttcAGCAAGTTCGGATCCAA
Ch10	SYN35158	ACGTTGGATGCCACACGGCATGTTTCAAAG	ACGTTGGATGAAATACCCCTCTCTCCGTC	ggagATAGCTCAGAGCTGCTGGAAT
Ch10	SYN36092	ACGTTGGATGAAAATCCAAGCTGGGCGCA	ACGTTGGATGGTAGAACCGCTCGATGAGAG	ggacCCGCTCGATGAGAGCAATGA
Ch10	SYN11610	ACGTTGGATGGTTGTCATGCGTTGCTGATG	ACGTTGGATGCAACCATGGAGCATTTGCTG	TCCCCTCCCCTTCTC

SNP ID refers to the marker name in Illumina MaizeSNP50 BeadChip. The three primer sequences for each SNP assay are listed in 5'to 3'order.

Table 3: List of 301 SNPs that map equivalently well to more than one physical location within the B73 RefGen_v4 sequence.

SNP Identifier	Inclusion status	Nature of Duplication	Bp distance between BLAST hits
SYN10525	Included in set of 6,745 SNPS	Two positions physically linked	19,108
SYN10530	Included in set of 6,745 SNPS	Two positions physically linked	19,108
SYN13768	Included in set of 6,745 SNPS	Two positions physically linked	19,715
SYN14938	Included in set of 6,745 SNPS	Two positions physically linked	17,720
SYN19948	Included in set of 6,745 SNPS	Two positions physically linked	30,809
SYN24655	Included in set of 6,745 SNPS	Two positions physically linked	24,378
SYN19951	Included in set of 6,745 SNPS	Two positions physically linked	30,810
SYN23940	Included in set of 6,745 SNPS	Two positions physically linked	23,666
SYN94	Included in set of 6,745 SNPS	Two positions physically linked	117,884
SYN23936	Included in set of 6,745 SNPS	Two positions physically linked	23,666
SYNGENTA16435	Included in set of 6,745 SNPS	Two positions physically linked	23,666
PUT-163a-93012953-4804	Included in set of 6,745 SNPS	Two positions physically linked	16,336
PZE-101088646	Included in set of 6,745 SNPS	Two positions physically linked	19,479
PZE-101130533	Included in set of 6,745 SNPS	Two positions physically linked	19,834

Table 3: Continued

PZE-102111952	Included in set of 6,745 SNPS	Two positions physically linked	38,609
PZE-103034362	Included in set of 6,745 SNPS	Two positions physically linked	19,711
PZE-104018218	Included in set of 6,745 SNPS	Two positions physically linked	11,822
PZE-104019423	Included in set of 6,745 SNPS	Two positions physically linked	24,976
PZE-104058430	Included in set of 6,745 SNPS	Two positions physically linked	3,932,054
PZE-104153704	Included in set of 6,745 SNPS	Two positions physically linked	22,008
PZE-106005094	Included in set of 6,745 SNPS	Two positions physically linked	16,465
PZE-106005121	Included in set of 6,745 SNPS	Two positions physically linked	16,466
PZE-106064721	Included in set of 6,745 SNPS	Two positions physically linked	20,854
PZE-106068094	Included in set of 6,745 SNPS	Two positions physically linked	17,493
PZE-107055266	Included in set of 6,745 SNPS	Two positions physically linked	23,762
PZA03063.21	Excluded from mapping set	Two positions physically linked	26,460
PZA03076.10	Excluded from mapping set	Two positions physically linked	213,332
PZB02542.1	Excluded from mapping set	Two positions physically linked	13,782
PZB02542.2	Excluded from mapping set	Two positions physically linked	14,276
PZB02544.1	Excluded from mapping set	Two positions physically linked	18,221
PZB02544.2	Excluded from mapping set	Two positions physically linked	18,545
PZB02544.3	Excluded from mapping set	Two positions physically linked	17,897
SYN10523	Excluded from mapping set	Two positions physically linked	19,108
SYN10527	Excluded from mapping set	Two positions physically linked	19,108
SYN10529	Excluded from mapping set	Two positions physically linked	19,108
SYN10531	Excluded from mapping set	Two positions physically linked	19,108
SYN24654	Excluded from mapping set	Two positions physically linked	24,378
SYN24656	Excluded from mapping set	Two positions physically linked	24,378
SYN25218	Excluded from mapping set	Two positions physically linked	18,499
SYN25219	Excluded from mapping set	Two positions physically linked	18,499
SYN29264	Excluded from mapping set	Two positions physically linked	15,753
SYN31200	Excluded from mapping set	Two positions physically linked	1,254,242
SYN37981	Excluded from mapping set	Two positions physically linked	15,599

Table 3: Continued

SYN37985	Excluded from mapping set	Two positions physically linked	15,600
SYN37987	Excluded from mapping set	Two positions physically linked	15,599
SYN37988	Excluded from mapping set	Two positions physically linked	15,600
SYN6035	Excluded from mapping set	Two positions physically linked	25,620
SYN6036	Excluded from mapping set	Two positions physically linked	25,620
SYN6041	Excluded from mapping set	Two positions physically linked	25,620
SYN7847	Excluded from mapping set	Two positions physically linked	30,808
ZM002904-0519	Excluded from mapping set	Two positions physically linked	26,781
ba1.5	Excluded from mapping set	Two positions physically linked	19,344
ba1.7	Excluded from mapping set	Two positions physically linked	19,344
PHM8549.4	Excluded from mapping set	Two positions physically linked	85,728
PZA02977.4	Excluded from mapping set	Two positions physically linked	48,920
PZB02547.1	Excluded from mapping set	Two positions physically linked	22,708
PZD00033.3	Excluded from mapping set	Two positions physically linked	3,990
SYN10535	Excluded from mapping set	Two positions physically linked	19,108
SYN10536	Excluded from mapping set	Two positions physically linked	19,108
SYN10537	Excluded from mapping set	Two positions physically linked	19,108
SYN10958	Excluded from mapping set	Two positions physically linked	79,964
SYN10980	Excluded from mapping set	Two positions physically linked	17,108
SYN19952	Excluded from mapping set	Two positions physically linked	30,810
SYN23937	Excluded from mapping set	Two positions physically linked	23,666
SYN23939	Excluded from mapping set	Two positions physically linked	23,666
SYN24040	Excluded from mapping set	Two positions physically linked	9,661
SYN25223	Excluded from mapping set	Two positions physically linked	18,499
SYN26088	Excluded from mapping set	Two positions physically linked	15,249
SYN95	Excluded from mapping set	Two positions physically linked	117,884
SYNGENTA16437	Excluded from mapping set	Two positions physically linked	23,666
ZM012429-0380	Excluded from mapping set	Two positions physically linked	18,499
ba1.6	Excluded from mapping set	Two positions physically linked	19,344

Table 3: Continued

SYN10984	Excluded from mapping set	Two positions physically linked	17,108
PZA00493.2	Excluded from mapping set	Two positions physically linked	33,220
PZA03100.4	Excluded from mapping set	Two positions physically linked	22,728
PUT-163a-50332421-2218	Excluded from mapping set	Two positions physically linked	22,939
PUT-163a-76293556-3972	Excluded from mapping set	Two positions physically linked	70,622
PUT-163a-78122842-4411	Excluded from mapping set	Two positions physically linked	24,294
PUT-163a-148954015-533	Excluded from mapping set	Two positions physically linked	19,811
PUT-163a-71766778-3515	Excluded from mapping set	Two positions physically linked	18,499
PUT-163a-28986612-1722	Excluded from mapping set	Two positions physically linked	17,554
PUT-163a-60397057-2952	Excluded from mapping set	Two positions physically linked	5,590,120
PZA00486.2	Excluded from mapping set	Two positions physically linked	749,894
PUT-163a-71766778-3512	Excluded from mapping set	Two positions physically linked	18,499
PUT-163a-18167798-1299	Excluded from mapping set	Two positions physically linked	26,459
PZE-101029990	Excluded from mapping set	Two positions physically linked	19,108
PZE-101029991	Excluded from mapping set	Two positions physically linked	19,108
PZE-101044748	Excluded from mapping set	Two positions physically linked	29,583
PZE-101099503	Excluded from mapping set	Two positions physically linked	4,284
PZE-101102440	Excluded from mapping set	Two positions physically linked	186,112,779
PZE-101114902	Excluded from mapping set	Two positions physically linked	4,645
PZE-101115581	Excluded from mapping set	Two positions physically linked	7,231
PZE-101116556	Excluded from mapping set	Two positions physically linked	22,415
PZE-101120408	Excluded from mapping set	Two positions physically linked	28,881
PZE-101120411	Excluded from mapping set	Two positions physically linked	28,881
PZE-101120413	Excluded from mapping set	Two positions physically linked	28,881
PZE-101130497	Excluded from mapping set	Two positions physically linked	19,811
PZE-101130500	Excluded from mapping set	Two positions physically linked	19,811
PZE-101133399	Excluded from mapping set	Two positions physically linked	1,770,792
PZE-101140640	Excluded from mapping set	Two positions physically linked	22,158

Table 3: Continued

PZE-101241431	Excluded from mapping set	Two positions physically linked	198,951
PZE-101244564	Excluded from mapping set	Two positions physically linked	22,568
PZE-102024868	Excluded from mapping set	Two positions physically linked	26,713
PZE-102039358	Excluded from mapping set	Two positions physically linked	20,026
PZE-102039367	Excluded from mapping set	Two positions physically linked	20,021
PZE-102039378	Excluded from mapping set	Two positions physically linked	19,992
PZE-102054921	Excluded from mapping set	Two positions physically linked	21,542
PZE-102069683	Excluded from mapping set	Two positions physically linked	7,199
PZE-102076145	Excluded from mapping set	Two positions physically linked	22,163
PZE-102076225	Excluded from mapping set	Two positions physically linked	196,899
PZE-102090146	Excluded from mapping set	Two positions physically linked	8,516
PZE-102100867	Excluded from mapping set	Two positions physically linked	19,581
PZE-102100869	Excluded from mapping set	Two positions physically linked	19,581
PZE-102100870	Excluded from mapping set	Two positions physically linked	19,579
PZE-102100871	Excluded from mapping set	Two positions physically linked	19,579
PZE-102100879	Excluded from mapping set	Two positions physically linked	19,578
PZE-102102921	Excluded from mapping set	Two positions physically linked	33,409
PZE-102110009	Excluded from mapping set	Two positions physically linked	326,234
PZE-102112009	Excluded from mapping set	Two positions physically linked	186,816
PZE-102112011	Excluded from mapping set	Two positions physically linked	186,816
PZE-102113760	Excluded from mapping set	Two positions physically linked	15,599
PZE-102113762	Excluded from mapping set	Two positions physically linked	15,599
PZE-102113763	Excluded from mapping set	Two positions physically linked	15,599
PZE-102113765	Excluded from mapping set	Two positions physically linked	15,599
PZE-102113766	Excluded from mapping set	Two positions physically linked	15,599
PZE-102146692	Excluded from mapping set	Two positions physically linked	30,810
PZE-102146710	Excluded from mapping set	Two positions physically linked	30,809
PZE-102146884	Excluded from mapping set	Two positions physically linked	30,808
PZE-102153851	Excluded from mapping set	Two positions physically linked	185,552,371

Table 3: Continued

PZE-103062159	Excluded from mapping set	Two positions physically linked	15,297
PZE-103066547	Excluded from mapping set	Two positions physically linked	4,898
PZE-103098377	Excluded from mapping set	Two positions physically linked	13,786
PZE-103125724	Excluded from mapping set	Two positions physically linked	22,620
PZE-103158625	Excluded from mapping set	Two positions physically linked	14,218
PZE-103158628	Excluded from mapping set	Two positions physically linked	14,218
PZE-103158629	Excluded from mapping set	Two positions physically linked	14,218
PZE-103158635	Excluded from mapping set	Two positions physically linked	14,218
PZE-103158636	Excluded from mapping set	Two positions physically linked	14,218
PZE-103171654	Excluded from mapping set	Two positions physically linked	19,163
PZE-104000354	Excluded from mapping set	Two positions physically linked	17,437
PZE-104003833	Excluded from mapping set	Two positions physically linked	28,854
PZE-104035095	Excluded from mapping set	Two positions physically linked	271,274
PZE-104035979	Excluded from mapping set	Two positions physically linked	6,867
PZE-104041268	Excluded from mapping set	Two positions physically linked	56,051
PZE-104041273	Excluded from mapping set	Two positions physically linked	56,051
PZE-104057816	Excluded from mapping set	Two positions physically linked	23,604
PZE-104057817	Excluded from mapping set	Two positions physically linked	23,604
PZE-104058429	Excluded from mapping set	Two positions physically linked	14,448
PZE-104060779	Excluded from mapping set	Two positions physically linked	31,891
PZE-104060780	Excluded from mapping set	Two positions physically linked	31,893
PZE-104066937	Excluded from mapping set	Two positions physically linked	4,684
PZE-104067721	Excluded from mapping set	Two positions physically linked	435,640
PZE-104092702	Excluded from mapping set	Two positions physically linked	4,772
PZE-104120535	Excluded from mapping set	Two positions physically linked	20,143
PZE-104132015	Excluded from mapping set	Two positions physically linked	16,700
PZE-104132033	Excluded from mapping set	Two positions physically linked	16,695
PZE-104132035	Excluded from mapping set	Two positions physically linked	16,695
PZE-104132036	Excluded from mapping set	Two positions physically linked	16,695

Table 3: Continued

PZE-104143197	Excluded from mapping set	Two positions physically linked	7,681
PZE-104143198	Excluded from mapping set	Two positions physically linked	7,681
PZE-105030596	Excluded from mapping set	Two positions physically linked	57,257
PZE-105038816	Excluded from mapping set	Two positions physically linked	21,431
PZE-105045416	Excluded from mapping set	Two positions physically linked	9,712
PZE-105046456	Excluded from mapping set	Two positions physically linked	25,688
PZE-105046457	Excluded from mapping set	Two positions physically linked	25,688
PZE-105046466	Excluded from mapping set	Two positions physically linked	25,693
PZE-105068397	Excluded from mapping set	Two positions physically linked	15,871
PZE-105068404	Excluded from mapping set	Two positions physically linked	15,874
PZE-105077741	Excluded from mapping set	Two positions physically linked	12,251
PZE-105080680	Excluded from mapping set	Two positions physically linked	3,384
PZE-105088859	Excluded from mapping set	Two positions physically linked	298,057
PZE-105093927	Excluded from mapping set	Two positions physically linked	15,101
PZE-105097904	Excluded from mapping set	Two positions physically linked	11,549
PZE-105105974	Excluded from mapping set	Two positions physically linked	65,678
PZE-105108663	Excluded from mapping set	Two positions physically linked	3,747
PZE-105110675	Excluded from mapping set	Two positions physically linked	34,636
PZE-106005120	Excluded from mapping set	Two positions physically linked	16,465
PZE-106005130	Excluded from mapping set	Two positions physically linked	16,467
PZE-106005295	Excluded from mapping set	Two positions physically linked	5,144
PZE-106012919	Excluded from mapping set	Two positions physically linked	160,020
PZE-106013484	Excluded from mapping set	Two positions physically linked	7,281
PZE-106014445	Excluded from mapping set	Two positions physically linked	26,459
PZE-106014450	Excluded from mapping set	Two positions physically linked	26,459
PZE-106029431	Excluded from mapping set	Two positions physically linked	28,742
PZE-106029434	Excluded from mapping set	Two positions physically linked	28,742
PZE-106031737	Excluded from mapping set	Two positions physically linked	14,265,679
PZE-106035753	Excluded from mapping set	Two positions physically linked	82,197

Table 3: Continued

PZE-106053251	Excluded from mapping set	Two positions physically linked	27,855
PZE-106058019	Excluded from mapping set	Two positions physically linked	21,260
PZE-106064720	Excluded from mapping set	Two positions physically linked	20,854
PZE-107006271	Excluded from mapping set	Two positions physically linked	60,736
PZE-107019024	Excluded from mapping set	Two positions physically linked	11,907
PZE-107038442	Excluded from mapping set	Two positions physically linked	16,918
PZE-107039786	Excluded from mapping set	Two positions physically linked	26,209
PZE-107039787	Excluded from mapping set	Two positions physically linked	26,209
PZE-107055301	Excluded from mapping set	Two positions physically linked	23,762
PZE-107055306	Excluded from mapping set	Two positions physically linked	23,762
PZE-107055363	Excluded from mapping set	Two positions physically linked	23,764
PZE-107057730	Excluded from mapping set	Two positions physically linked	4,627
PZE-107065680	Excluded from mapping set	Two positions physically linked	7,272
PZE-107066232	Excluded from mapping set	Two positions physically linked	25,450
PZE-107094282	Excluded from mapping set	Two positions physically linked	41,553
PZE-107113482	Excluded from mapping set	Two positions physically linked	16,338
PZE-107113484	Excluded from mapping set	Two positions physically linked	16,340
PZE-107128264	Excluded from mapping set	Two positions physically linked	18,500
PZE-108009197	Excluded from mapping set	Two positions physically linked	7,640
PZE-108009198	Excluded from mapping set	Two positions physically linked	7,640
PZE-108018598	Excluded from mapping set	Two positions physically linked	17,113
PZE-108039859	Excluded from mapping set	Two positions physically linked	3,596
PZE-108054876	Excluded from mapping set	Two positions physically linked	779,946
PZE-108059579	Excluded from mapping set	Two positions physically linked	1,483
PZE-108070212	Excluded from mapping set	Two positions physically linked	3,122
PZE-108088324	Excluded from mapping set	Two positions physically linked	7,451
PZE-108088583	Excluded from mapping set	Two positions physically linked	28,258
PZE-109018122	Excluded from mapping set	Two positions physically linked	64,451
PZE-109018135	Excluded from mapping set	Two positions physically linked	64,451

Table 3: Continued

PZE-109027987	Excluded from mapping set	Two positions physically linked	5,188
PZE-109047268	Excluded from mapping set	Two positions physically linked	7,822
PZE-109078789	Excluded from mapping set	Two positions physically linked	111,387
PZE-109085323	Excluded from mapping set	Two positions physically linked	17,287
PZE-109122183	Excluded from mapping set	Two positions physically linked	20,315
PZE-110008968	Excluded from mapping set	Two positions physically linked	186,262
PZE-110013362	Excluded from mapping set	Two positions physically linked	10,328
PZE-110029058	Excluded from mapping set	Two positions physically linked	17,560
PZE-110031648	Excluded from mapping set	Two positions physically linked	21,179
PZE-110031869	Excluded from mapping set	Two positions physically linked	13,326
PZE-110042836	Excluded from mapping set	Two positions physically linked	54,509
PZE-110045687	Excluded from mapping set	Two positions physically linked	7,791
PZE-110053171	Excluded from mapping set	Two positions physically linked	3,484
PZE-110054216	Excluded from mapping set	Two positions physically linked	42,623
PZE-110054219	Excluded from mapping set	Two positions physically linked	42,623
PZE-110054222	Excluded from mapping set	Two positions physically linked	42,623
PZE-110066556	Excluded from mapping set	Two positions physically linked	15,216
PZE0005896632	Excluded from mapping set	Two positions physically linked	12,535
PUT-163a-16919246-1009	Excluded from mapping set	Two positions physically linked	70,622
PZE-110045686	Excluded from mapping set	Two positions physically linked	7,791
PZA03423.2	Excluded from mapping set	Two positions physically linked	47,120
PZE-108018447	Excluded from mapping set	Two positions physically linked	16,710
PZE-105084722	Excluded from mapping set	Two positions physically linked	38,197,044
PZE-107042637	Excluded from mapping set	Two positions physically linked	2,139,123
PZE-103062160	Excluded from mapping set	Two positions physically linked	15,297
PUT-163a-18167798-1300	Excluded from mapping set	Two positions physically linked	26,459
PZE-108028342	Excluded from mapping set	Two positions physically linked	23,160
PZE-106014622	Excluded from mapping set	Two positions physically linked	8,000
PZE-107047192	Excluded from mapping set	Two positions physically linked	4,976

Table 3: Continued

PUT-163a-74235050-3609	Excluded from mapping set	Two positions physically linked	192
PUT-163a-18179900-1492	Excluded from mapping set	Two positions physically linked	1,576
PZA00335.12	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZB01358.3	Excluded from mapping set	Positions are on separate chromosomes	N/A
SYN29249	Excluded from mapping set	Positions are on separate chromosomes	N/A
SYN29736	Excluded from mapping set	Positions are on separate chromosomes	N/A
ZM010606-0698	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZB01446.1	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZA03313.3	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZB01728.7	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-101030089	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-101046118	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-101114075	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-101119095	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-101129348	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-101134693	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-101168178	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-101178435	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-101178446	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-101178451	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-101198749	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-101198753	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-102021588	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-102021627	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-102052125	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-102130469	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-102130490	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-103045672	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-103062559	Excluded from mapping set	Positions are on separate chromosomes	N/A

Table 3: Continued

PZE-103062560	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-103091187	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-103102547	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-104066292	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-105045422	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-105084486	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-106021921	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-106106877	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-107025740	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-107044256	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-108044116	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-108070743	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-109022899	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-109041540	Excluded from mapping set	Positions are on separate chromosomes	N/A
PUT-163a-29576931-1795	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-102086077	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-108028396	Excluded from mapping set	Positions are on separate chromosomes	N/A
ZM013386-0381	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-108035141	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-103059837	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-102090251	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-108035138	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-110063324	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-107065684	Excluded from mapping set	Positions are on separate chromosomes	N/A

Table 3: Continued

PUT-163a-78122849-4414	Excluded from mapping set	Positions are on separate chromosomes	N/A
PUT-163a-71318546-3146	Excluded from mapping set	Positions are on separate chromosomes	N/A
PZE-110025518	Excluded from mapping set	Positions are on separate chromosomes	N/A

Only 25 of these SNPs remained in the mapping set after filtering, reflecting a disproportionately poor retention rate likely due to performance problems of their assays. A vast majority of the cases reflect tandem duplications, with several examples of loose physical linkage.

Table 4: Numerical comparisons of numbers and spacings of SNPs between the unmasked and masked versions of the BMsyn10DHL maps built using 247 DHLs.

	Unmasked				**Masked**			
[a]Chromosome	[b]Number of markers	[c]Length (cM)	[d]Average spacing (cM)	[e]Max spacing (cM)	[b]Number of Markers	[c]Length (cM)	[d]Average spacing (cM)	[e]Max spacing (cM)
1	1259	533.0	0.4	8.2	727	259.6	0.4	4.0
2	809	387.2	0.5	7.3	506	226.4	0.4	7.4
3	600	329.3	0.5	6.2	471	216.4	0.5	6.2
4	806	492.1	0.6	5.7	447	200.9	0.5	4.5
5	712	397.7	0.6	11.8	433	172.0	0.4	11.2
6	615	308.9	0.5	6.7	327	149.2	0.5	6.6
7	577	321.0	0.6	19.0	346	177.2	0.5	17.0
8	544	297.1	0.5	15.3	352	174.7	0.5	14.8
9	446	211.4	0.5	4.1	323	147.3	0.5	4.1
10	377	187.2	0.5	5.1	259	130.4	0.5	4.6
overall	6745	3464.9	0.5	19.0	4191	1854.1	0.4	17.0

[a]Chromosomes
[b]Number of markers on each chromosome
[c]Length of each chromosome in CentiMorgan (cM)
[d]Average spacing of the markers on each chromosome in CentiMorgan (cM)
[e]Maximum spacing of markers on each chromosome in CentiMorgan (cM)

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CHAPTER 3. GENETIC ANALYSIS OF QUANTITATIVE DISEASE RESISTANCE AGAINST TWO ISOLATES OF NORTHERN CORN LEAF BLIGHT (*setosphaeria turcica*)

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Abstract

Plant diseases account for more than 40% of crop losses. Disease resistance is the most effective means of protecting crops against diseases. However, the absence of functional *R*-genes against fungal diseases in some crops and its instability in others resulting from the breakdown of *R*-gene mediated resistance has necessitated research in quantitative disease resistance (QDR) mechanisms with the goal of breeding for durable plant protection. Importantly, mechanisms of resistance should be broadly effective against various populations of pathogens. We took on both phenotypic and genetic approaches to understand the mechanisms that underlie QDR in maize-*Setosphaeria turcica* (two isolates) as a model for plant-hemibiotroph QDR interactions.

Intermated B73xMo17 Doubled Haploid lines (IBMDHLs) were evaluated for resistance to two isolates of *Setosphaeria turcica* in four years and two locations (Aurora, NY 2011 and 2012 and Boone, IA 2014 and 2015). Disease severity was measured using area under disease progress curve (AUDPC) and the IBMDHL population offered higher marker density and power for genetic mapping. Disease severity phenotypes varied across genotypes and environments resulting in 18 unique environment specific and 5 environment non-specific disease related quantitative trait loci (QTL). Genetic effects varied across locations with Boone accounting for larger QTL effects than Aurora. Previously reported and new putative quantitative resistance loci (QRL) were identified on all chromosomes except chromosome 10. Two genes reported to confer resistance to northern and southern leaf blight overlapped with a QTL on chromosome 1 in our study. Eleven confidence interval regions reported to colocalize with genes associated with disease resistance in previous studies were detected and could be hot-spots for disease resistance loci. This study highlights and provides a deeper appreciation of the polygenic control of northern leaf blight (NLB) resistance and the relationship between quantitative and qualitative resistance. Novel QTL that can be characterized and advanced for breeding of NLB resistant germplasm were identified.

Key words: *Setosphaeria turcica*, maize, quantitative disease resistance, quantitative trait loci, Intermated B73xMo17 Doubled Haploid lines, area under disease progress curve, northern leaf blight

Background

Global population is estimated to increase to 9.8 billion by 2050 and 11.2 billion by 2100 (UN DESA report, 2017). This puts pressure on increasing agricultural production by 70% (FA0, 2009), especially maize whose demand is projected to increase by 60% by the year 2050 (http://bdspublishing.com/_webedit/uploaded-Sfiles/All%20Files/Maize%20blog%20key%20challenges.pdf). Like all crops, maize production is constrained by its ability to respond to abiotic factors such as adverse weather conditions, soil characteristics, as well as biotic factors including: insect pests, pathogens and competition from weeds (Shiferaw et al. 2011). Biotic factors lead to a 48-54% decrease in maize yield (Shiferaw et al. 2011). The extent of yield loss depends on the host, environmental conditions and the pathogen.

Setosphaeria turcica teleomorph *Exserohilum turcicum* is a hemi-biotrophic fungus that causes northern leaf blight in maize (Condon et al. 2013; Flaherty and Dunkle 2005; Xue et al. 2013). During periods of high humidity and moderate temperatures (15-25^{o}C), *S. turcica* mycelia penetrate the leaf epidermal layer and grow into the xylem tissue where they secrete Helminthosporium turcicum (HT) toxin (Jennings, & Ullstrup, 1957). The symptoms of successful fungal invasion of a susceptible host are cigar shaped lesions on leaves which eventually extend to maize stalks, shanks and ears (Nebraska Extension NebGuide G2270). Northern corn leaf blight results in greater yield losses in early than in late maturing susceptible hybrids (Galiano-Carneiro and Miedaner 2017).

A variety of management practices that include biological control (Sartori et al. 2015), fungicides (Roberto Luis De et al. 2015; Robertson and Pecinovsky 2016), reduced tillage and multiple cropping (Sumner et al. 1981) and use of resistant varieties have been used to decrease

the adverse effects of northern corn leaf blight (Galiano-Carneiro and Miedaner 2017)). Host-plant resistance remains the most effective and affordable technique for reducing yield losses resulting from diseases (Mundt 2014). Host-plant resistance is divided into qualitative and quantitative forms. Qualitative resistance is controlled by major single, mostly race-specific resistant genes (R-genes) (Galiano-Carneiro and Miedaner 2017). Several R-genes have been identified that protect against *S. turcica,* including *Ht1, Ht2, Ht3, Htn1, Htm1* and *HtP* (Balint-Kurti et al. 2010; Nelson et al. 2018; Welz and Geiger 2000). Over time, the durability of these *Ht* genes has been minimized by host-pathogen evolution and changes in environmental conditions contributing to reduced effectiveness of qualitative resistance (Galiano-Carneiro and Miedaner 2017). Loss of durability and race-specificity of the *Ht R*-genes was demonstrated by Weems & Bradley (2018). 156 *S.turcica* isolates that were collected in 13 years from seven states of the US were race tested on maize lines carrying *Ht1, Ht2, Ht3, Htm1* and *Htn1 R*-genes. 47% of the isolates caused disease in the presence of multiple *Ht* R-genes while an average of 33% isolates were virulent to individual *Ht R*-genes (Weems and Bradley 2018). Quantitative resistance is conversely more durable, controls a wider range of pathogen races and is minimally affected by the environment (Carson and Vandyke 1994). Quantitative disease resistance (QDR) leads to a reduction of disease rather than a complete absence of disease. QDR is controlled by many genes with minor effects (Boyd et al. 2013; Burdon et al. 2014; Niks et al. 2015; Poland et al. 2009; Young 1996) involved in basal defense, regulation of morphology and development, detoxification, and defense signal transduction (Fukuoka et al. 2009; Krattinger et al. 2009; Kushalappa et al. 2016; Manosalva et al. 2009; Niks et al. 2015; Poland et al. 2009). Significant progress has been made in understanding the genetic architecture of QDR in plant pathogen interactions (Glazier et al. 2002; Welz et al. 1999; Wisser et al. 2005) leading to

identification of quantitative trait loci (QTL) in rice (Danesh et al. 1994; Wang et al. 1994), tomatoes (Danesh et al. 1994; Wang et al. 1994) potato (Leonards-Schippers et al. 1994) and maize (Bubeck et al. 1993). Additionally, researchers have identified QTLs for resistance to *Setosphaeria turcica* in different maize populations (Chung et al. 2010; Li et al. 2018; Welz and Geiger 2000; Wisser et al. 2006; Wisser et al. 2008). (Welz et al. 1999) identified 19 QTLs in their $F_{2:3}$ maize populations generated from resistant and susceptible parents**.** Additionally, a study by (Poland et al. 2011) yielded 29 QTLs for NLB resistance in a nested association mapping population. Interestingly, there is considerable variation in the number of NLB resistance QTLs present in each population. The importance of genetic diversity of the populations used for such studies was highlighted in the review by (Nelson et al. 2018). To capitalize on the diversity of QDR mechanisms, our group conducted QTL analysis in an intermated B73 x Mo17 doubled haploid (IBMDHL) population. This population was created by randomly intermating B73 x Mo17 siblings for ten generations followed with doubled haploidization providing higher resolution for genetic mapping (Hussain et al. 2007). The differences in reaction of B73 and Mo17 to NLB (Balint-Kurti et al. 2010; Balint-Kurti et al. 2007) also makes it an ideal population for this study.

Besides genetic background, variation of phenotypic traits is strongly affected by environmental conditions (Johana et al. 2017; Li et al. 2018). The study by (Thakur et al. 1989) revealed the effect of genetic background and changes in environmental conditions on the functionality of the *Htn1* locus that confers quantitative resistance to NLB. Consequently, testing genotypes in multiple environments and identifying QTLs that are consistently expressed across these environments is a vital aspect for evaluating durability of QDR (Galiano-Carneiro and Miedaner 2017; Goudemand et al. 2013; Hamon et al. 2011; Nelson et al. 2018). The goal of

this study was to broaden our understanding of the genetic mechanisms of QDR in the maize-*S. turcica* interaction. We used QTL analysis to identify genomic regions associated with resistance to northern corn leaf blight in a highly recombinant fixed population. Additionally, analyzing these lines in multiple environments using multiple NLB isolates allowed us to assess stability and specificity of the identified QTLs.

Materials and Methods

Fungal isolates and inoculum preparation

The Iowa Northern corn leaf blight fungal isolate *(NLB-IA01)* was provided by Dr. Alison Robertson at Iowa State University while the New York Isolate (*NLB-NY01*) was provided by Dr. Rebecca Nelson's lab group at Cornell University. NLB inoculum was prepared as described by (Chung et al. 2010) with minor modifications respective to the location where the field experiments were conducted (Iowa and New York). Briefly, single spores of each fungal isolate were isolated onto separate potato dextrose agar (PDA) plates and cultured at room temperature with no light for seven days, or until the PDA plates were fully colonized by the fungus. Meanwhile, sorghum grain was immersed in water for ~12 hours, drained, and placed into spawn bags with filter patch (1L per bag). Spawn bags containing grain were then autoclaved (using the liquid cycle) for a period of two hours for two consecutive days. Fully colonized PDA was removed from the plastic plates, cut into small pieces, and distributed among the spawn bags containing autoclaved sorghum. The bags were then maintained in the laboratory at room temperature and an 8:16 hour photoperiod (light: darkness) for approximately 14 days to allow fungal colonization of the sorghum. During this incubation, the inoculated sorghum mixture was stirred every two days to encourage uniform colonization of the grains. Once

colonization was complete, the sorghum was spread out to dry for 2-3 days; once fully dry, the sorghum was transferred to clean, dry containers and stored until field inoculation.

Plant materials

The maize population used in this study was acquired from Pioneer-HiBred. The Intermated B73XMo17 Doubled Haploid lines (IBMDHLs) were developed by crossing B73 (PI 550473) with Mo17 (PI 558532), followed by randomly mating siblings for 10 subsequent generations. This increased the number of recombination events and enlarged the genetic map which together improved genetic resolution (Hussain et al. 2007). Following ten generations of random mating, the lines underwent doubled haploidization, resulting in fixed highly recombinant lines (Holloway et al. 2011; Hussain et al. 2007). The two parental lines, Mo17 and B73 are relatively resistant and relatively susceptible (respectively) to NLB (Balint-Kurti et al. 2010; Balint-Kurti et al. 2007) which makes the IBMDHLs an ideal population for studying the genetic basis for quantitative resistance to NLB. Parental lines B73 and Mo17 and IBMDHLs were grown and evaluated for resistance to NLB in Aurora, NY (385 lines), and Boone, IA (330 lines).

Experimental design and inoculation

Field experiments were conducted in two locations, Aurora Research Farm in Aurora, New York, USA and the Agricultural Engineering and Agronomy Research Farm in Boone, Iowa, USA. A replicated randomized complete block design was executed in two years constituting four trials; NY-2011, NY-2012, IA-2014 and IA-2015. In Aurora, NY, 3.05m long single-row plots representing individual lines were replicated twice. Each row consisted of 15 plants evenly spaced with 0.20m between plants after overplanting and subsequent thinning. Row spacing was 0.76m between rows along the long axis. End-to-end row separation was

0.76m. Field experiments in Aurora, NY were conducted in 2011 and 2012 using 385 IBMDHLs inoculated with *NLB-NY01*, a New York NLB isolate. Experiments in Boone, IA were conducted in 2014 and 2015 using 330 IBMDHLs inoculated with *NLB-IA01*, an NLB isolate from Iowa. In both years, two complete block replications of 330 single rows were planted. Each plot was 4.57m in length and contained 18 plants after overplanting and subsequent thinning, using a minimum between-plant spacing of 0.20m. Spacing between rows was 0.76m along the long axis, and 0.91m in the end-to-end orientation. The two parents B73 and Mo17 were used as checks for all the field experiments. Double row border plots surrounded each field in both locations.

Plants were manually inoculated with local NLB isolates in each location/year. Individual plants were inoculated by placing approximately eight NLB colonized dry sorghum grains in the leaf whorl at V6-V7 stage. Inoculation was conducted shortly before it rained in both locations improving conditions for spore germination.

Rating and phenotypic data collection

In all four trials, disease severity was scored on scale of 0 to 100% (Pataky et al. 1998) for necrotic (diseased) leaf area (DLA). The lower percentages signified resistance and higher percentages signified susceptibility. Visual evaluation and scoring of individual plots was conducted at one week intervals starting from two weeks after onset of anthesis in NY and at V8 in Iowa. Three ratings were taken each year in Aurora, and six ratings were taken each year in Boone.

Statistical analyses

Disease severity was calculated as the mean of the ratings for each of the four trials using area under disease progress curve (AUDPC) as described in (Balint-Kurti et al. 2010; Balint-Kurti et al. 2007; Tekeoglu et al. 2000).

AUDPC was calculated as: $Y = \sum [(X_i + X_{i+1})/2](t_{i+1} - t_i)$

where Y is AUDPC, X_i is the blight score of the ith evaluation, X_{i+1} is the blight score of the $i + 1^{th}$ evaluation, and $(t_{i+1} - t_i)$ is the number of days between two evaluations. AUDPC per day was calculated by dividing AUDPC by the total number of days that disease severity was evaluated. Best linear unbiased predictions (BLUPs) of the AUDPC per day values were calculated for each line using the R package lme4 (Douglas et al. 2015) for each environment to eliminate environmental effects. Analysis of variance (ANOVA) was used to analyze the phenotypic data (AUDPC) for the two locations (Aurora, NY and Boone, IA). R package (V. 3.5.0) was used to compute least square means between replicates in each environment taking into consideration the 6.4% missing data in one replicate in both years of Aurora. JMP software was used to calculate phenotypic Pearson correlation coefficients. Below is the linear model that was used for the two years within each location

$$Y_{ijk} = \mu + G_i + E_j + GE_{ij} + R(E)_{jk} + \varepsilon_{ijk}$$

where Y_{ijk} denotes AUDPC per day of the ith Genotype within the k_{th} replication within the jth Environment, μ is the overall mean, G_i is the effect of the ith Genotype, E_j is the effect of the jth Environment, Genotype × Environment$_{ij}$ is the effect of the interaction between the ith Genotype and the jth Environment, $R(E)_{jk}$ is the effect of the k_{th} replication within the jth Environment, and

ε_{ijkl} is the residual effect. Environment signifies year effect (2011 and 2012 in Aurora; 2014 and 2015 in Boone) and replicate signifies block effect since each replicate was a single block. Environment, genotype, replicate in each environment and their interactions were considered as random factors. Estimates of heritability the linear mixed-effects model (LMM) were calculated using lmer of lme4 package (R V.3.5.0) (Hallauer et al. 2010; Janick 2003).

Heritability was calculated using;

For individual environments (years), H^2 was computed as:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \left(\frac{\sigma_e^2}{r}\right)}$$

Across environments (years), H^2 was computed as:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{e} + \frac{\sigma_e^2}{er}}$$

where σ^2_g denotes genetic variance, σ^2ge denotes genotype x environment(year) interaction, e denotes number of environments (years) , σ^2_e denotes residual variance and r is the number of replications (Hallauer et al. 2010).

Genetic linkage map construction

IBMDHLs were genotyped by Pioneer-HiBred using the Illumina MaizeSNP50 BeadChip. Physical positions of single nucleotide polymorphism (SNPs) markers were determined by BLAST (basic local alignment search tool) results of SNP flanking sequences to the B73 RefGen_V4. BLAST-hits with an e-value less than 1e-10 were selected and their positions recorded (Ganal et al. 2011). Markers were excluded from the analysis based on data quality (e.g. high percentage of missing data) and usefulness (e.g. monomorphic). A total of 4,191 physically positioned SNPs were considered for genetic map construction. Excluding

genotypes with an increased percentage of missing marker data and heterozygous calls, 247 genotypes and 4,191 SNPs were used for constructing a linkage map covering 1854.1cM using JoinMap4 (Stam 1993). The Haldane mapping function was used to calculate genetic distances (centiMorgans) between the markers ordered by their physical position (Collard et al. 2005). Average and maximal distance between markers was 0.4cM and 17cM respectively.

Quantitative trait loci analysis

Because genotype by environment interaction had significant effects on NLB severity, QTL analysis of the least square mean AUDPC in individual trials (Boone, IA:- 2014 and 2015 and Aurora, NY:- 2011 and 2012) was performed using composite interval mapping of the R/qtl package (R V 3.5.0) (Broman and Sen 2009). Composite interval mapping was performed for individual years. Using the forward selection method, six significant markers were selected as cofactors (Broman and Sen 2009). Scanning of QTL was done with a step size of 1cM excluding cofactors found within 3cM of the scanned region. Neighboring QTL with LOD peaks at a distance greater than 20cM were considered separate QTL.

Results

Evaluation of Northern corn leaf blight resistance across the IBMDHL population

The IBMDHLs and the two parents (B73 and Mo17) were evaluated in two locations (Boone, IA and Aurora, NY). For each location, a local isolate (*NLB-IA01* from Iowa and *NLB-NY01* from New York) was used for inoculation. Overall, 385 and 330 IBMDHLs were screened in Aurora, NY and Boone, IA respectively. Each line was visually scored based on disease symptoms using a scale that ranged from of 0-100%. Disease severity for the two years for each location was calculated using area under disease progress curve (AUDPC). AUDPC per day was calculated by dividing AUDPC by the total number of days that disease severity was evaluated.

AUDPC per day values were adjusted using best linear unbiased predictions (BLUPs) to eliminate environmental effects. These results represent 329 lines common in Boone and Aurora.

Variation in disease severity was observed among the lines and ranged from 3%- 50% with an average of 19% in Aurora, 2011. In 2012, Aurora had an average of 27% with a range of 3.5%-62%. In 2014, an average of 52% and a range of 17%-78% was measured in Boone and an average of 34% with a range of 9%-63% was measured in Boone, 2015. Disease severity was generally higher in Boone than in Aurora (Fig. 1).

All four locations exhibited relatively normal disease distributions, with B73 always being more susceptible to NLB compared to Mo17 (Fig. 2). B73 had an average of 33% and a range of 23% - 55% where as Mo17 had an average of 20% and a range of 8% - 43%. A proportion of IBMDLs exhibited lower disease severity scores while others had higher scores than the two parental lines. Notably, AUDPC per day distribution in Aurora was skewed more to the left side of the axis for both years. lines were categorized as resistant when their AUDPC per day value ranged between 0-40%. Therefore, it is not surprising that 88% and 97% of the IBMDHLs rated below 40% in Aurora, 2012 and 2011 respectively while 14% and 64% of the IBMDHLs rated below 40% in Boone, 2014 and 2015 respectively (Fig. 2).

Disease severity increased as the growing season progressed and was measured as AUDPC. Correlation coefficients between replicates within trials ranged between r = 0.60 and r = 0.70 in Aurora and r = 0.79 and r = 0.92 in Boone (Table 1). Correlation coefficients within and between locations were highly significant (P<0.001) and moderately high (r = 0.62 to r = 0.82) (Table 2).

AUDPC per day values were used to categorize IBMDHLs into 3 groups as follows: resistant (0-40%), moderately resistant (41-69%) and susceptible (70-100%) (Fig. 3).

Comparison of average disease severity scores across years and locations yielded groups of unique and shared IBMDHLs that had the lowest AUDPC per day values. Aurora, 2011 and 2012 had 32 and 2 unique resistant IBMDHLs with low NLB severity scores respectively. Apart from one line, all the lines that exhibited NLB resistance in Boone were also resistant in Aurora. Forty two lines were resistant against both isolates in all four environments (Fig. 3).

Since a combined analysis of variance for the two locations showed significant environmental effects, separate analyses were conducted for each location (Aurora, NY and Boone, IA). Genotype, year and genotype-by-year interaction effects were significant in both locations at p-value <0.001. However, year accounted for the highest variance in both locations (Table 3). A high broad sense heritability of 88% and 92% was obtained for individual environments and all four environments respectively.

Genotyping and QTL analysis

QTL analysis a powerful statistical method for determining the genetic basis of complex traits (Kearsey 1998; Li et al. 2010). High marker density map offered by the IBMDHL population and the availability of B73 and Mo17 sequenced genomes (Lai et al. 2010; Schnable et al. 2009) provided unique sources of genetic information (MaizeGDB.org) from which polymorphisms underlying NLB resistance were identified. Two hundred and forty seven lines were used in the QTL analysis. Mean AUDPC correlations were moderately high within and between locations (data not shown). Due to the significant ($P<0.01$) effect of genotype by environment interaction on disease severity, separate QTL analysis was conducted for individual years (trials). Table 5 shows chromosomal positions of SNP markers flanking QTL regions in centimorgan (cM), physical positions of markers flanking QTL regions in base pairs (bp, B73 RefGen_V4), additive (a) effect of the QTL (peak) and R^2 estimating the proportion of

phenotypic variance explained by the identified QTL. Traits are average AUDPC values in each of the four years in Aurora and Boone locations (AUDPC2011AU, AUDPC2012AU, AUDPC2014BN, and AUDPC2015BN). Asterisks in Table 5 show instances where QTLs were detected in more than one trial. The cutoff threshold for determining a significant marker was set at a logarithm of odds (LOD) score of 2.4 and a significance level of $P \leq 0.05$ (Table 5). Number of markers mapped on each chromosome varied with an average spacing of 0.4cM-0.5cM, and was proportional to the length of the chromosome.

QTLs were detected in all four trials at an experiment-wise significance threshold of $\alpha = 0.05$. QTLs for NLB resistance were found on all chromosomes except 10 (Table 5). The percentage of NLB resistance variance explained by detected QTLs ranged from 0.5% to 9.8%. Five QTLs overlapped within and across locations on chromosomes 1, 4, 6, 7 and 9. Four of the 5 overlapping QTL were detected across the two locations (Chromosome 1, 4, 6 and 7) while the overlapping QTL on chromosome 9 was identified in the two Iowa trials (2014 and 2015). Four QTL uniquely detected in 2011AU accounted for 4.7% to 7.1% of the phenotypic variance in NLB resistance. Five QTL accounting for 1.3% to 6.3% of the phenotypic variance were detected in 2012AU. Moreover, 6 and 3 QTL accounting for 0.5% to 7.8% and 2.6% to 9.4%, respectively, were uniquely detected in 2014BN and 2015BN respectively. QTL effect magnitudes for |additive genetic effect| ranged from 33 to 186.42 AUDPC trait units. B73, the more susceptible parent decreases NLB in 8 QTL on chromosomes 1,2,3,4,6 and 9, and increases it in 15 QTL on chromosomes1,2,4,5,6,7 and 8 (Table 5).

We identified 4 QTL regions on chromosomes 1, 2, 6 and 8 that were previously reported to colocalize with receptor-like kinases (RLK) (GRMZM2G136513, GRMZM2G424908, GRMZM2G079082, GRMZM2G082112, GRMZM2G164612, GRMZM2G169584 and

GRMZM2G169571) (Li et al. 2018). Moreover, RLK such as nucleotide binding leucine rich repeat (NB-LRR) are plant proteins that detect pathogen virulence factors in gene-for-gene plant-pathogen interactions (Deyoung and Innes 2006; Greeff et al. 2012). Notably, GRMZM2G136513, GRMZM2G424908 and GRMZM2G164612 have been reported to have an association with resistance to NLB (Li et al. 2018) and therefore their chromosomal location might be a hot spot for NLB resistance genes. Furthermore, GRMZM2G017629 and GRMZM2G438824 were previously associated with resistance to Northern and Southern leaf blight (Li et al. 2018).

Discussion

Durable disease resistance is attributable to a group of multiple minor effect genes (Parlevliet, 2002). The challenge faced in identifying genes controlling quantitative traits can be managed by using a population that has a high density of markers because it offers a high resolution for identification of these genes. The IBMDHL population used in this study has a high marker density and offers higher resolution and power for genetic mapping (Hussain et al. 2007; Liu et al. 2015; Ma et al. 2018) compared to biparental populations previously used in similar studies.

Variation in disease severity was high across the IBMDHLs, between locations and across years within each location (Fig. 1). The differences observed in our trials were attributed to variation in environmental conditions and the NLB isolates used. Disease development across lines was greater in Boone especially in 2014 than in Aurora (Fig. 2). Additionally, only 17% of the lines categorized as resistant exhibited resistance phenotypes across all four trials and could be potential sources of superior non-specific NLB resistance. The observed variation in disease severity could be attributable to differences in weather conditions (data not shown) since growth

and development of *Setosphaeria turcica* is affected by temperature (59 to 77°F) and humidity (Galiano-Carneiro and Miedaner 2017; Levy and Cohen 1983; Levy and Pataky 1992). Aurora received an average rainfall of 1.0 inch in 10 events and the average temperature ranged between 58°F and 78°F during the growing season in 2011, and 1.0 inch of rainfall in eight events with the average temperature between 58°F to 79°F during the growing season in 2012. On the other hand, Boone received an average of 2.0 inches of rainfall in 11 events and the average temperature was between 60°F -79°F during the growing season in 2015. The average rainfall received in Boone was twice as much as that received in Aurora. It is also possible that differences in disease severity between locations were caused by differences in *Setosphaeria turcica* isolates used to inoculate plants in Iowa (*NLB-IA01*) versus New York (*NLB-NY01*). Similarly, Balint-Kurti et al., (2010) reported differences in disease pressure across intermated B73 X Mo17 (IBM) population tested in Clayton, NC and Aurora, NY.

Disease severity scores were consistent between replications within each trial (year) (Table 1), between trials within and across locations (Table 2). Broad-sense heritability was high for all experiments conducted. These two observations suggest a strong genetic control of NLB resistance. Moreover genotype and environment are major contributors to phenotypic variance. The impact of genotype and environment on NLB resistance was evident in our study. Year, genotype, and year by genotype interactions accounted for variation in disease severity with year accounting for the highest proportion of the variance (Table 3). This is in agreement with previous studies that reported the effect of genotype and environment on phenotypic variance (Balint-Kurti et al. 2010; Li et al. 2018). Li et al., (2018) tested a panel of nested association mapping (NAM) population in the United States (US) and China and reported environment and genotype impact on resistance to southern and northern leaf blight. Similarly, (Balint-Kurti et al.

2010) reported genotype and environment as the major factors affecting NLB resistance in the IBM population which was tested in Clayton, NC and Aurora, NY. Variation in disease severity suggests differences in resistance or resistance mechanisms both of which could be a good resource for studying the genetic mechanisms underlying QDR and the specificity of quantitative resistance-associated loci.

Our study identified 23 QTL on chromosomes 1, 2, 3, 4, 5, 6, 7, 8 and 9 (Table 5). A large proportion of the QTL identified explained a moderate percentage (R^2 <10%) of the phenotypic variation. Eighteen of the 23 QTL identified were environment specific while the other 5 QTL overlapped between locations and years. However, the additive effects were different across locations with Boone accounting for larger QTL effects than Aurora (Table 5). These larger QTL effects in Boone than Aurora support the higher disease levels in Boone than Aurora. Genes underlying NLB resistance- related QTL had substantial additive genetic effects of 33 to 186.42. IBMDHLs carrying two B73 alleles at Chr01:cM76.7-81.51 averaged 372.84 AUDPC units of additional disease symptoms compared to lines carrying two Mo17 alleles at the same locus in 2015 (Table 5). Identification of the same QTL across environments was not surprising considering the high significant correlations between years and locations shown in table two. It is possible that these QTL are independent of isolate and, or environment differences, a great characteristic to consider when selecting and breeding for disease resistance across multiple environments. However, some QTL exhibited isolate and environment specificity as they were only identified in individual trials (Table 5). It will be interesting to assess the reaction of the IBMDHL population to the two NLB isolates under similar environmental conditions.

This study identified 11 confidence interval regions that have previously been reported to have an association with disease resistance (Table 6) (Li et al. 2018; Poland et al. 2011). Poland et al.(2009) conducted a 3 year NLB study using the NAM population in Aurora, NY. Li et al. (2018) also screened the NAM population for NLB and SLB resistance in the US and China. Both studies identified QTL near or within chromosomal regions that have been implicated in disease resistance. QTL regions on chromosomes 1, 4, 5, 8 and 9 colocalized with genes that have plant defense associated characteristics such as phytoalexin production and antifreeze mechanisms, contributing to disease resistance (Poland et al. 2011). QTL on chromosome 1 (PZE-101085909-PZE-101090113) from our study colocalized with AC214524.3_FG002 that has a phytochrome P450 domain (Poland et al. 2011). An antifreeze protein (GRMZM2G173771) colocalized with a chromosome 4 QTL (PZE-104100613-PZE-104102755) identified in our study. Antifreeze properties are reported to be exhibited by some pathogenesis-related (PR) proteins during qualitative disease resistance (Griffith and Yaish 2004; Poland et al. 2011). Marker interval 139,700,146 to 165,267,318 on chromosome 8 colocalizes with genes that have domains with several disease resistance related characteristics for example GRMZM2G014089 an ATPase/ABC transporter containing domain.

Marker confidence intervals that had multiple disease resistance associated QTL or genes could be hot spots for identification of resistance genes and genes that confer resistance to multiple diseases. Co-localization of QTL identified in our study with previously reported disease resistance associated QTL is evidence that quantitative resistance loci (QRL) could be weaker forms of major resistance (R) genes as hypothesized by (Poland et al. 2009). Unique QTL associated with NLB resistance were also mapped and these could be capitalized on to identify novel resistance genes. This study also highlighted the polygenic control of NLB

resistance in maize and the range of mechanisms that might be underlying QDR as seen in Table 6. Additionally, results from this study support environment and or isolate specificity of QDR as shown by the greater number of QTL identified in specific trials (18) than overlapping QTL(5) (Table5).

Figures and Tables

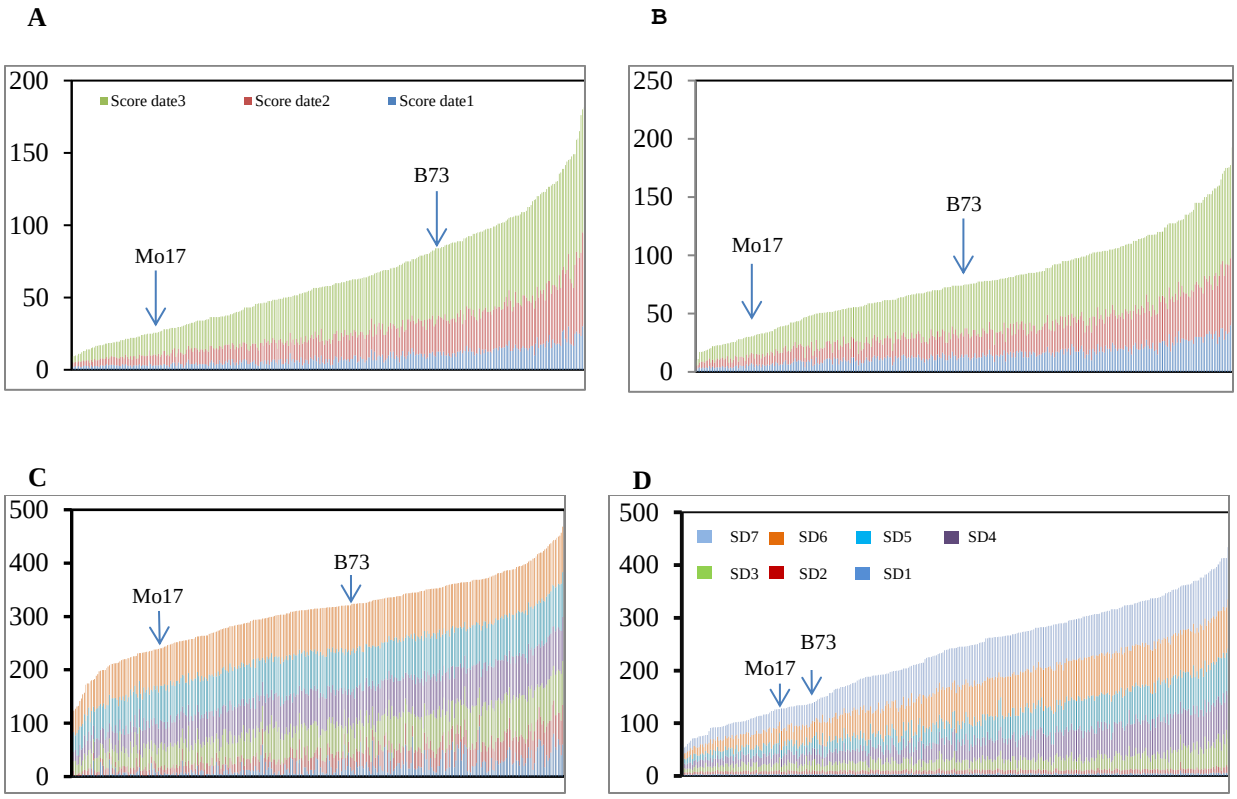

Figure 1: Variation of cumulative average northern leaf blight severity scores measured in the intermated B73 x Mo17 doubled-haploid lines across 3 score dates in (**A**) 2011 and (**B**) 2012 Aurora, NY, and across 6 and 7 score dates in (**C**) 2014 and (**D**) 2015 Boone, IA respectively. Parental lines Mo17 and B73 are indicated with blue arrows.

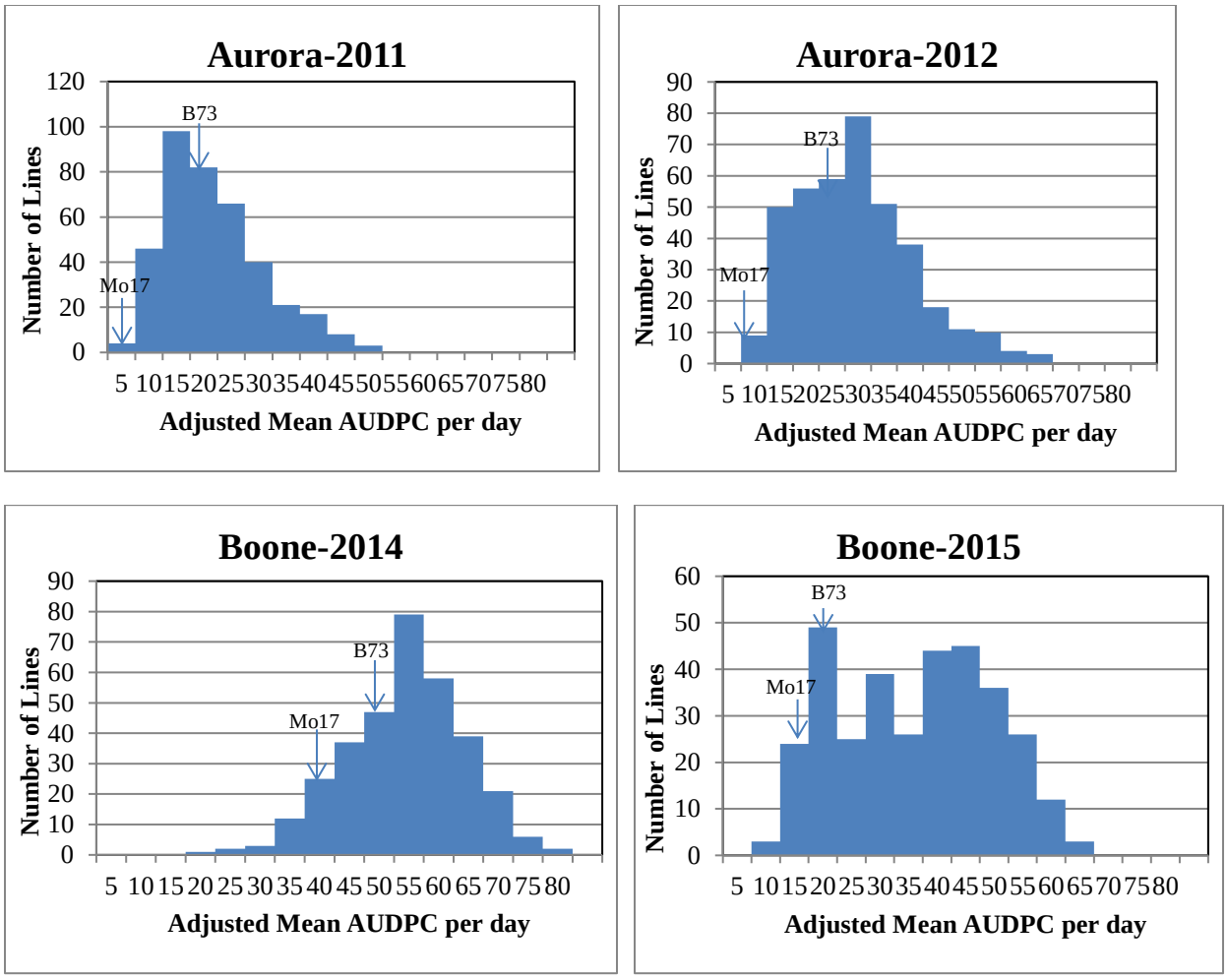

Figure 2: Frequency distribution of mean AUDPC per day measured in the intermated B73 x Mo17 doubled-haploid lines across 3 score dates in Aurora, NY (2011 and 2012) and across 6 and 7 score dates in Boone, IA 2014 and 2015 respectively. Parental lines Mo17 and B73 are indicated with blue arrows.

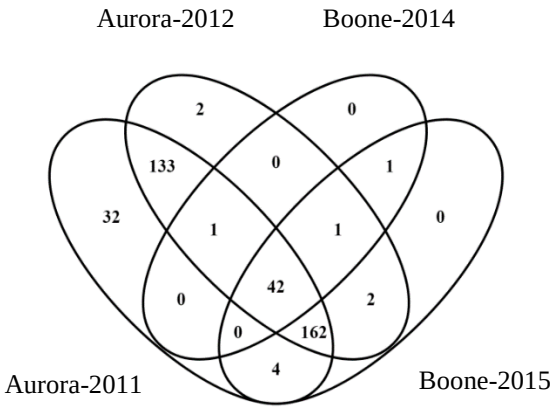

Figure 3: Unique and overlapping resistant IBMDHLs in Aurora, NY (2011 and 2012) and Boone, IA (2014 and 2015)

Table 1: Pearson correlation coefficients for northern leaf blight scores between replications in within trials

	Rep1Aurora11	Rep1Aurora12	Rep1Boone14	Rep1Boone15
Rep2Aurora11	0.60***			
Rep2Aurora12		0.70***		
Rep2 Boone 14			0.79***	
Rep2 Boone 15				0.92***

Disease scores were averaged across 3 score dates in Aurora, NY (2011 and 2012) and 6 and 7 score dates in Boone, IA 2014 and 2015 respectively. Significant at ***p < 0.001

Table 2: Pearson correlation coefficients of northern leaf blight scores within and between locations

	Aurora12	Boone 14	Boone 15
Aurora11	0.82***	0.62***	0.71***
Aurora12		0.66***	0.76***
Boone14			0.79***

Disease scores were averaged across 3 score dates in Aurora, NY (2011 and 2012) and 6 and 7 score dates in Boone, IA 2014 and 2015 respectively. Significant at ***p < 0.001

Table 3: Analysis of variance for northern corn leaf blight disease scores across IBMDHLs in Aurora, 2011 and 2012; and Boone, 2014 and 2015.

Location	Factors	DF	Mean Square	F value	*P* value
Aurora(2011 & 2012)	Genotype	325	542.7	10.1477	< 2.2e-16***
	Year	1	19407	362.9222	< 2.2e-16***
	Genotype*Year	325	67.2	1.2576	0.008017**
	Rep(Year)	2	1286.4	24.0554	8.57e-11***
	Residual	629	53.5		

Table 3: Continued

Boone(2014 & 2015	Genotype	325	598	23.1575	< 2.2e-16***
	Year	1	105541	4090.4631	< 2.2e-16***
	Genotype*Year	325	93	3.5931	< 2.2e-16***
	Rep(Year)	2	1220	47.2725	< 2.2e-16***
	Residual	650	26		

Significant at ***p<0.001 and **p<0.001

Table4: Allocation of markers on each of the 10 maize chromosomes

[a]Chromosome	[b]# markers	[c]Length (cM)	[d]Average spacing (cM)	[e]Max spacing (cM)
1	727	259.6	0.4	4.0
2	506	226.4	0.4	7.4
3	471	216.4	0.5	6.2
4	447	200.9	0.5	4.5
5	433	172.0	0.4	11.2
6	327	149.2	0.5	6.6
7	346	177.2	0.5	17.0
8	352	174.7	0.5	14.8
9	323	147.3	0.5	4.1
10	259	130.4	0.5	4.6
overall	4191	1854.1	0.4	17.0

[a]Chromosomes
[b]Number of markers on each chromosome
[c]Length of each chromosome in CentiMorgan (cM)
[d]Average spacing of the markers on each chromosome in CentiMorgan (cM)
[e]Maximum spacing of markers on each chromosome in CentiMorgan (cM)

Table 5: Quantitative trait loci in 247 IBMDHLs underlying resistance against *NY01* and *IA01* NLB isolates across four trials: Aurora, NY (2011, 2012) and Boone, IA (2014, 2015)

[a]Trait	[b]Flanking Markers	[c]Chromosome	[d]LOD	[e]Genetic_ Interval (cM)	[f]2-LOD Interval (bp)	[g]a	[h]R^2
AUDPC2011AU	SYN27959-SYN32085	1	2.86	190.77-194.47	257,870,704-261,049,714	55	4.72
AUDPC2011AU	SYN2021-SYN21640	1*	4.50	243.51-246.7	298,581,657-299,603,790	-49	3.69
AUDPC2011AU	SYN364-PZE-101029820	1	4.66	51.32-55.32	17,164,290-18,154,825	59	5.67
AUDPC2011AU	PZE-104146082-SYN28888	4*	3.91	162.55-166.47	239,625,361-240,980,164	48	3.48
AUDPC2011AU	SYN2354-SYN33747	5	6.04	3.67-7.25	1,763,389-2,417,012	67	7.16
AUDPC2011AU	PZE-108079510-SYN32654	8	3.86	87.67-104.53	139,700,146-165,267,318	56	5.08
AUDPC2012AU	PZE-101085909-PZE-101090113	1	3.97	102.39-104.98	77,338,211-83,410,999	71	5.57
AUDPC2012AU	PZE-102060229-PZE-102068688	2	2.48	90.46-98.49	40,117,453-48,537,928	55	3.66
AUDPC2012AU	SYN5902-PZE-102176644	2	3.07	178.78-183.63	223,448,315-226,081,587	33	1.33
AUDPC2012AU	PZE-102163927-SYN24423	2	4.20	160.45-163.64	215,841,861-217,174,089	-55	
AUDPC2012AU	SYN13507-SYN22086	6*	2.51	41.59-45.91	100,106,884-100,890,021	-55	3.48
AUDPC2012AU	SYN12256-SYN17012	7*	3.41	133.09-137.44	173,657,054-174,828,719	61	4.44
AUDPC2012AU	PZE-109057210-PZE-109064132	9	6.65	69.12-73.00	101,778,026-109,487,798	-72	6.29
AUDPC2014BN	SYN9135-SYN12170	1	6.79	84.13-87.72	40,784,327-47,788,739	123	7.81
AUDPC2014BN	SYN15181-PZE-103036157	3	3.07	74.78-78.55	21,835,478-29,230,414	-94	4.4
AUDPC2014BN	PZE-104025973-PZE-104029141	4	4.37	73.58-78.01	32,691,436-36,839,190	82	3.57
AUDPC2014BN	SYN4986-PZE-106077771	6	2.77	72.63-76.48	133,965,077-137,026,119	30.82	0.51
AUDPC2014BN	PZE-106042027-SYN2959	6	6.91	33.00-36.40	95,983,083-95,983,275	-87	4.09

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Table 5: Continued

AUDPC2014BN	SYN12256-PZE-107128348	7*	2.49	133.09-139.07	173,657,054-176,159,739	81	3.57
AUDPC2014BN	PZE-108005219-PZE-108008403	8	2.44	21.80-30.99	5,543,710-8,758,454	118	6.97
AUDPC2014BN	SYN9773-SYN38163	9*	7.17	83.96-89.23	136,093,954-138,441,052	-135	8.84
AUDPC2015BN	SYN2420-PZE-101145571	1	3.91	129.08-133.37	185,326,108-190,421,572	160.2	9.36
AUDPC2015BN	PZE-101248317-SYN8230	1*	4.42	244.99-248.73	299,058,261-300,808,633	-131.65	5.02
AUDPC2015BN	PZE-101050744-PZE-101053027	1	4.15	76.7-81.51	35,300,185-37,285,375	186.42	9.80
AUDPC2015BN	SYN11059-PZE-104149997	4*	2.56	158.90-170.65	238,093,107-241,790,217	152	6.39
AUDPC2015BN	PZE-104100613-PZE-104102755	4	3.53	118.42-122.45	180,322,530-181,788,697	-94.63	2.60
AUDPC2015BN	PZE-106048958-PZE-106051387	6*	3.18	43.93-47.64	100,780,261-105,515,198	-159.57	7.55
AUDPC2015BN	PZE-109085150-SYN38163	9*	3.32	86.09-89.23	136,350,618-138,441,052	-179.65	8.64

[a]Individual traits are represented by average area under disease progress curve (AUDPC) which is a measure of disease severity followed by year and location in which experiments were conducted

[b]Markers bordering the 2-LOD interval within which the QTL lies

[c]Chromosome on which the QTL is located

[d]Logarithm of odds (LOD) score of the position of maximum likelihood of the QTL.

[e]CentiMorgan (cM) positions of the marker interval within which the highest likelihood for the QTL lies

[F]Base pair physical position (B73_RefGen_V4) of the marker interval within which the highest likelihood for the QTL lies

[g]a – additive genetic effect estimate of the QTL in terms of average AUDPC. Positive and negative additive values are indicative of B73 allele contributing to disease increase and Mo17 allele contributing to decreased disease severity

[h]R^2 - estimate of phenotypic variance (%) explained by a single QTL

*Asterisks - instances where QTLs were detected in more than one trial

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Table 6: Colocalization of known disease resistance-related genes with NLB resistance-related QTL from our study

[a]Trait	[b]Chromosome	[c]Flanking Markers	[d]Genetic_ Interval (cM)	[e]Physical_ interval (B73 RefGen_V4) (bp)	[f]LOD	[g]Gene ID	[j]Physical_ position (B73 RefGen_V4) (bp)	[k]Domains/ biological process
AUDPC2015BN	1	PZE-101050744-PZE-101053027	76.7-81.51	35,300,185-37,285,375	4.2	[h]GRMZM2G136513 (Li et al. 2018)	36,588,522	CC-NB-LRR
AUDPC2012AU	1	PZE-101085909-PZE-101090113	102.39-104.98	77,338,211-83,410,999	4.0	AC214524.3_FG002 (Poland et al. 2011)	79,506,307	Cytochrome P450
AUDPC2015BN	1	SYN2420-PZE-101145571	129.08-133.37	85,326,108-190,421,572	3.9	GRMZM2G113840 (Poland et al. 2011)	185,876,713	Cellular retinaldehyde binding/alpha-tocopherol transport
						GRMZM2G080652 (Poland et al. 2011)	185,977,739	Mov34/MPN/ PAD-1
						GRMZM2G142597 (Poland et al. 2011)	189,580,903	RNA recognition motif
						GRMZM2G441903 (Poland et al. 2011)	189,593,205	Zinc finger,
						[i]GRMZM2G017629 (Li et al. 2018)	186,945,218	Similar to disease resistance response protein

Table 6: Continued

						[i]GRMZM2G438824 (Li et al. 2018)	187,013,825	Similar to disease resistance protein
AUDPC2011AU-AUDPC2015BN	1	SYN2021-SYN8230	243.51-248.73	298,581,657-300,808,633	4.5	[h]GRMZM2G424908 (Li et al. 2018)	300,433,186	NB-LRR
AUDPC2012AU	2	PZE-102060229-PZE-102068688	90.46-98.49	40,117,453-48,537,928	2.5	GRMZM2G131448 (Poland et al. 2011)	47,414,810	NLB resistance
AUDPC2012AU	2	PZE-102163927-SYN24423	160.45-163.64	215,841,861-217,174,089	4.2	GRMZM2G079082 (Li et al. 2018)	215,971,156	CC-NB-LRR
AUDPC2015BN	4	PZE-104100613-PZE-104102755	118.42-122.45	180,322,530-181,788,697	3.5	GRMZM2G079381 (Poland et al. 2011)	181,552,730	Nitrite/sulphite reductase, hemoprotein beta-component, ferrodoxin-like
						GRMZM2G126646 (Poland et al. 2011)	180,558,735	Helix-turn-helix motif, lambda-like repressor
						GRMZM2G173771 (Poland et al. 2011)	181,560,436	Antifreeze protein, type I, Pathogenesis-related transcriptional factor and ERF, DNA-binding
AUDPC2011AU	5	SYN2354-SYN33747	3.67-7.25	1,763,389-2,417,012	6.0	GRMZM2G398668 (Poland et al. 2011)	1,940,928	5'-3' exonuclease, DNA repair protein

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Table 6: Continued

						GRMZM2G069203 (Poland et al. 2011)	160,278,127	Bacterial transferase hexapeptide repeat, Serine O-acetyltransferase, Serine acetyltransferase, N-terminal
AUDPC2012AU-AUDPC2015BN	6	SYN13507-PZE-106051387	41.59-47.64	100,106,884 -105,515,198	3.2	GRMZM2G082112 (Li et al. 2018)	101,154,908	NB-LRR
AUDPC2011AU	8	PZE-108079510-SYN32654	87.67-104.53	139,700,146-165,267,318	3.9	[h]GRMZM2G164612 (Li et al. 2018)	156,763,311	RLK
						GRMZM2G311680 (Poland et al. 2011)	155,473,422	Peptidase A1, Ribosomal protein P2, Peptidase aspartic
						GRMZM2G014089 (Poland et al. 2011)	157,385,847	ATPase, AAA+ type, ABC transporter
						GRMZM2G010987 (Poland et al. 2011)	157,447,787	UDP-glucuronosyl/UDP-glucosyltransferase
						GRMZM2G145019 (Poland et al. 2011)	159,028,132	Mitochondrial carrier protein, Adenine nucleotide translocator 1
						GRMZM2G169584 (Li et al. 2018)	160,338,061	CC-NB

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Table 6: Continued

						GRMZM2G169571 (Li et al. 2018)	160,377,553	CC-NB
						GRMZM2G138995 (Li et al. 2018)	161,301,289	ABC-type transport system involved in resistance to organic solvents
						GRMZM2G167872 (Li et al. 2018)	161,705,740	Zea mays clone 243057 bifunctional polymyxin resistance arnA protein mRNA
AUDPC2014BN	9	SYN9773-SYN38163	83.96-89.23	136,093,954-138,441,052	7.2	GRMZM2G326783 (Poland et al. 2011)	136,494,931	DNA-binding

[a]Individual traits are represented by average area under disease progress curve (AUDPC), a measure of disease severity followed by year and location in which experiments were conducted
[b]Chromosome on which the QTL is located
[c]Markers bordering the 2-LOD interval within which the QTL lies
[d]CentiMorgan (cM) positions of the marker interval within which the highest likelihood for the QTL lies
[e]Base pair physical position (B73 RefGen_V4) of the marker interval within which the highest likelihood for the QTL lies
[f]The logarithm of odds (LOD) score of the position of maximum likelihood of the QTL
[g]Gene name of known disease resistance related genes
[h]Gene implicated in NLB resistance
[i]Gene implicated in NLB and SLB resistance
[j]Base pair physical position (B73 RefGen_V4) of known disease resistance related genes
[k]Domains and biological properties of known disease resistance-related gen

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CHAPTER 4. CHARACTERIZING THE GENETIC MODE OF ACTION OF QUANTITATIVE DISEASE RESISTANCE ALLELES IN THE MAIZE-NORTHERN LEAF BLIGHT PATHOSYSTEM

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Abstract

Understanding the mechanism of gene action underlying multigenic loci that confer quantitative disease resistance is essential for developing highly resistant crop genotypes. We used North Carolina design III to develop two backcross populations from an intermated B73 x Mo17 doubled haploid (IBMDH) population. Quantitative trait loci (QTLs) mapping was conducted on IBMDH, B73 and Mo17 backcross hybrid (BC_{B73} and BC_{Mo17}) populations to identify major QTLs responsible for resistance of maize to northern corn leaf blight (NLB). Symptoms of NLB were measured on a 0-100% scoring scale. Disease scores were taken weekly for 7 weeks following manual inoculation with *Setosphaeria turcica,* the NLB fungus. Disease severity for IBMDH, BC_{B73} and BC_{Mo17} populations was calculated using area under disease progress curve (AUDPC).

The two BC_{F1} populations had significantly lower disease severity compared to the IBMDH population. We identified 26 QTLs related to quantitative NLB resistance and they spanned 9 of the 10 maize chromosomes. The highest number of NLB resistance-related QTLs were identified on chromosome 1. An estimation of the additive, dominant and over-dominant gene effects in the QTLs underlying NLB resistance revealed a higher number of over-dominant loci compared to complete dominant effect loci. This observation suggests the crucial impact of over-dominant loci in heterosis for NLB resistance.

Key words: Quantitative disease resistance, gene action, intermated B73xMo17 doubled haploid lines, North Carolina design III, quantitative trait loci, northern corn leaf blight, area under disease progress curve

Background

Most agronomically important traits including yield, and resistance to biotic and abiotic stresses are quantitatively inherited (Holland 2007; St.Clair 2010; Xing et al. 2002). Quantitative traits are controlled by multiple genes with small effects (Holland 2007; Xing et al. 2002). Single locus gene actions include additive, dominant, or additive by dominant epistatic interactions. Additionally, multiple locus gene actions include additive by additive and dominant by dominant epistatic interactions (Acquaah 2007; Li et al. 2010). Not only is unravelling the genetic basis of these multigenic traits key to improving genetically controlled traits, but also is determining the nature and extent of gene effects (Li et al. 2010; Mistry et al. 2016). Therefore, having genetic models and experimental designs that have a high likelihood of detecting gene effects is important (Melchinger et al. 2008).

North Carolina design I, II, III, biparental mating, diallel and polycross are some of the major mating designs that have been used to identify gene actions and measure their effects on quantitative trait loci (QTLs) underlying these multigenic traits (Acquaah 2007; Hallauer et al. 2010). NC-Design III is the most powerful mating design and when combined with analysis of variance (ANOVA), different genetic variance components are estimated with great precision (Comstock & Robinson, 1952, Mather & Jinks, 1982, (Cockerham and Zeng 1996). NC-Design III is used to study gene actions at multiple loci underlying quantitative traits. Moreover, under the assumption that linkage and epistasis are absent, the degree of the effect of additive and dominance components of variance on the quantitative trait being assessed can be determined (Hallauer et al. 2010; Li et al. 2010). These advantages have supported the use of NC-Design III in identifying QTLs that underlie complex traits (Frascaroli et al. 2007; Garcia et al. 2008; Kearsey et al. 2003; Kusterer et al. 2007; Schön et al. 2010; Xiao et al. 1995). The experimental units in NC-Design III are produced by crossing two inbred lines to produce an F_1 population followed by selfing the F_1 hybrids to produce an F_2 population which is then crossed back to the two inbred parents from which the F_1 population was derived (Acquaah 2007; Cockerham and Zeng 1996). The ratio of additive and dominance effects that is estimated from ANOVA provides the average level of dominance of the genes being tested (Cockerham and Zeng 1996; Garcia et al. 2008). Populations such as recombinant inbred lines (RILS) (Kusterer et al. 2007; Li et al. 2010), F_2 plants (Comstock and Robinson 1948), F_3 plants (Stuber et al. 1992) and F_7 populations (Xiao et al. 1995) have been used to unravel genetic effects of QTLs underlying heterosis. The fluidity of the heterozygous genetic nature of these populations limits their use in QTL analyses (Jiang et al. 2014; Mei et al. 2005).

Using doubled haploids is advantageous especially in self-pollinated plants in that the QTLs have fixed homozygous alleles (Hallauer et al. 2010; Mei et al. 2005; Smith et al. 2008).

Conversely, the lack of or limited heterozygosity that is the nature of such populations makes it challenging to analyze genetic effects that are non-additive (Mei et al. 2005). This is overcome by crossing permanently homozygous populations to parents with recessive or dominant alleles (Jiang et al. 2014; Luo et al. 2001; Mei et al. 2005) and immortalized F_2 populations (Hua et al. 2003) creating a heterozygous population for analysis.

My study used an intermated B73 x Mo17 doubled-haploid (IBMDH) backcross hybrid population. The IBMDH population is a maize population developed by 10 generations of intermating an F_2 population followed with doubled-haploidization which results in a high density marker map with high resolution and recombinant alleles that are fixed (Hussain et al. 2007; Liu et al. 2015; Ma et al. 2018). This population has been used to study genetic factors that control maize leaf cadmium accumulation (Zhao et al. 2018), biochemical and genetic factors that influence nitrogen metabolism in maize roots and leaves (Silva et al. 2018; Trucillo Silva et al. 2017), agronomic and genetic factors affecting cob related traits in different nitrogen systems (Jansen et al. 2015), and the QTLs underlying maize leaf structure (Ma et al. 2018).

To create backcross hybrid populations, the IBMDH lines were crossed to their parental lines, B73 and Mo17. The homozygous nature of doubled haploids (DHs) (Forster and Thomas 2005) makes them a unique population to create backcross hybrids, from which estimation of non-additive genetic effects of QTLs can be made. Furthermore, the genotype of each locus in the DH backcross hybrid is known because it results from a combination of fixed alleles from the DH parent and the recurrent parent lines (Li et al. 2010; Mei et al. 2005). The alleles of a given locus are homozygous if the DH and the recurrent parent are carrying the same alleles and heterozygous for the alternate locus (Mei et al. 2005). Following the North Carolina Design III mating scheme, 215 IBMDHLs were backcrossed to the two parental inbred lines (B73 and

Mo17) from which they descend to produce hybrids. NLB resistance was compared between doubled haploid lines and their backcross hybrids.

Materials and Methods

Plant materials

Three populations were used to dissect the gene actions that control QTLs underlying resistance to NLB. The intermated B73 x Mo17 doubled haploid lines (IBMDHLs) and the two back cross hybrid populations were obtained from Pioneer-HiBred. Population one is comprised of 215 IBMDHs developed by double haploidization of lines that had undergone 10 generations of random intermating an F_2 population from a cross between B73 (PI 550473) and Mo17 (PI 558532) (Fig. 1). This population has a genetic map that has a high density of markers which increases the precision for identifying QTLs, especially small effect QTLs associated with disease resistance (Holloway et al. 2011; Hussain et al. 2007). IBMDHLs were then crossed to their parental lines (B73 and Mo17), forming two backcross (BC) hybrid populations. Population two consisted of 215 BC hybrids developed from crosses between B73 (as the female) and the 215 IBMDHs. Population three consisted of 215 BC hybrids developed from crosses between Mo17 (as the female) and the 215 IBMDHs (Fig. 1). The two parents B73 and Mo17, the B73 x Mo17 hybrid and the Mo17 x B73 hybrid were used as checks. This field study was conducted at the Agronomy farm in Ames, IA. In total, 645 lines and hybrids plus checks were manually inoculated at the V6-V7 stage using a *Setosphaeria turcica* isolate from Iowa.

Experimental design

Field experiments were carried out during the summer months of 2015, 2016 and 2017 at the Agricultural Engineering and Agronomy Research Farm in Boone, Iowa, USA. IBMDHL and backcross hybrid populations were inoculated with *S. turcicum NLB-IA01* from Iowa. Plots

representing individual lines or BC hybrids were arranged in a randomized complete block design with two replicates, with each replicate representing a single block. Each plot was 4.57m in length and contained 18 plants after overplanting and subsequent thinning, using a minimum between-plant spacing of 0.20m. Spacing between rows was 0.76m along the long axis, and 0.91m in the end-to-end orientation. The field was surrounded by double row border plots. B73 and Mo17, the parental lines, as well as the hybrids B73 x Mo17, and Mo17 x B73 were included in each replicate as checks.

Fungal Isolates and Inoculum preparation

The *NLB-IA01* was obtained from Dr. Alison Robertson at Iowa State University. With a few modifications, fungal inoculum preparation was similar to the procedure described by (Chung et al. 2010). Briefly, individual fungal spores were isolated onto potato dextrose agar (PDA) plates and incubated at room temperature in the dark to allow colony formation. Sorghum grains were immersed in water for ~12 hours, drained, and placed into spawn bags (www.mycosupply.com) with filter patch (1L sorghum per bag), followed by two rounds of autoclaving. After seven days or upon full fungal colonization of the PDA plates, NLB-colonized PDA was distributed amongst the spawn bags containing autoclaved sorghum grains. The sorghum + *S. turcica* mixture was maintained in the laboratory at room temperature and 8:16 hour photoperiod (light:darkness) for approximately 14 days, and was regularly mixed to ensure uniform fungal growth and colonization of the sorghum. After full colonization, the sorghum was spread out to dry. Individual plants were manually inoculated at V6-V7 by placing about eight grains of colonized sorghum in the leaf whorl.

Rating and phenotypic data collection

Disease severity for each row representing either a line or a hybrid was scored once a week for the two month period following manual inoculation. Presence, size, and distribution of necrotic lesions was visually scored on a 0-100% scale (Pataky et al. 1998) with lower and higher percentages representing resistant and susceptible phenotypes, respectively.

Statistical analyses

Phenotypic data analysis

Because of unfavorable weather conditions for NLB development in 2016 and 2017, the data analyses only cover summer, 2015. Disease severity was measured using area under disease progress curve (AUDPC) (Balint-Kurti et al. 2010; Balint-Kurti et al. 2007; Tekeoglu et al. 2000).

AUDPC was calculated as: $Y = \sum [(X_i + X_{i+1})/2] (t_{i+1} - t_i)$

where Y represents AUDPC, X_i represents the disease score of the ith evaluation, X_{i+1} represents disease score of the $i + 1^{th}$ evaluation, and $(t_{i+1} - t_i)$ represents the time difference between two evaluations. JMP software was used to calculate phenotypic Pearson correlation coefficients.

Gene actions

Additive and dominant gene actions were analyzed in an IBMDH back cross population in order to characterize genetic control of disease resistance in maize. Analysis of variance was used to compare the variance components of IBMDHLs (non-segregating) and their backcross hybrids (segregating). Means for individuals within a family were tested for homogeneity using least significant difference (LSD) (Gomez & Gomez, 1984) before pooling to represent mean data for a particular family. T-test was used to determine the significance of gene effects. Gene effects and components of variance are notated as described by (Hallauer et al. 2010); mean (m),

additive (a), dominance (d), additive variance (σ^2_A), dominance variance (σ^2_D), phenotypic variance (σ^2_P), environmental variance (σ^2_E) and genotypic variance (σ^2_G).

Gene effects were calculated as follows (Hallauer et al. 2010):

$a = (P_1P_1 – P_2P_2)/2$

$d = H_{MP} = BCF_1 – MP$

MP = (DH + Recurrent parent)/2

where P_1 represents recurrent parent 1, P_2 represents recurrent parent 2, H_{MP} is the mid-parent heterosis for disease resistance, BC_{F1} represents the mean trait value of the backcross hybrids and MP is the mid-parental trait value of the corresponding IBMDHL and recurrent parent (B73 or Mo17).

Genetic linkage map construction

IBMDHLs were genotyped by Pioneer-HiBred using the Illumina MaizeSNP50 BeadChip. Physical positions of single nucleotide polymorphism (SNP) markers were determined by BLAST (basic local alignment search tool) results of SNP flanking sequences to the B73 RefGen_V4. BLAST-hits with an e-value less than 1e-10 were selected and their positions recorded (Ganal et al. 2011). Markers were excluded from the analysis based on data quality (e.g. high percentage of missing data) and usefulness (e.g. monomorphic). A total of 4,191 physically positioned SNPs were considered for genetic map construction. A list of 247 genotypes and 4,191 SNPs were used for constructing a linkage map covering 1854.1centiMorgan (cM) using JoinMap4 (Stam 1993) after genotypes with a high proportion of heterozygous calls and missing marker data had been removed. Genetic markers were arranged according to their physical positions. Genetic distances (cM) between these markers were

calculated using the Haldane mapping function (Collard et al. 2005). Average and maximal distance between markers was 0.4cM and 17cM respectively.

Quantitative trait loci analysis

QTL analysis of the mean AUDPC in the IBMDH, BC_{B73} and BC_{Mo17} populations and H_{MP} values for B73 and Mo17 (Boone, IA:- 2014 and 2015 and Aurora, NY:- 2011 and 2012) was performed using composite interval mapping of the R/qtl package (R V 3.5.0) (Broman and Sen 2009). Composite interval mapping was performed for individual traits. Using the forward selection method, six significant markers were selected as cofactors (Broman and Sen 2009). Scanning of QTLs was done with a step size of 1cM excluding cofactors found within 3cM of the scanned region. Neighboring QTLs with LOD peaks at a distance greater than 20cM were considered separate QTLs.

Results

Response of IBMDH, BCF_{1B73} and BCF_{1Mo17} populations to NLB

To examine genetic variability of quantitative resistance to NLB, 215 IBMDHLs and the two backcross populations (BCF_{1B73} and BCF_{1Mo17}) were evaluated for three years (2015, 2016 and 2017) in Boone, IA. However, unfavorable weather conditions prevented development of disease in the experiments conducted in 2016 and 2017. The results presented are from one successful year (2015) of field study. Plants were manually inoculated using an Iowa NLB fungal isolate (*NLB-IA01*) and visually scored for NLB symptoms on a scale of 0-100%. Disease severity was calculated using area under disease progress curve (AUDPC).

Disease severity and mean score differences across populations increased as the growing season progressed (Fig. 2). Disease severity significantly varied ($p < 0.001$) across populations with the highest mean scores reported in the doubled-haploid population (Fig. 3). The IBMDH

population had a 36% and 30% increase in AUDPC compared to BC_{Mo17} and $BC_{B73,}$ respectively; indicating greater susceptibility of the doubled-haploid lines to NLB than the BC hybrids. BC_{Mo17} hybrids had a lower mean AUDPC value compared to BC_{B73} hybrids. However, disease severity scores between BC_{Mo17} and BC_{B73} populations were not significantly different ($p > 0.001$) (Fig. 3).

Disease scores were nearly normally distributed across the three populations (Fig. 4). The majority of the BC hybrids had lower ranges of AUDPC values while disease scores in IBMDHLs were more widely spread out. This was supported by 76% of the BC_{B73} and 87% of BC_{Mo17} hybrid populations being highly resistant with an AUDPC value ≤ 820. However, only 28% of the IBMDH population had an AUDPC value ≤ 820 (Fig. 4).

B73 and Mo17 were the recurrent parents for the two backcross hybrid populations, and performance for each BC hybrid population was measured using mid-parent heterosis for NLB resistance (Jiang et al. 2014). BC_{Mo17} had the lowest AUDPC mean value (652.4±15), followed by BC_{B73} with 744±24 and lastly IBMDH lines with the highest AUDPC mean of 1275.1±50 (Table 1). The mean AUDPC values were generally higher for B73 populations (B73 parent - 928 and BC_{B73} - 744) compared to Mo17 populations (Mo17 - 752.3 and BC_{Mo17} - 652.4). B73 x Mo17 and Mo17 x B73 AUDPC means were not significantly different ($P>0.001$). The hybrid breakdown (HB) value of the IBMDH population (434.6) was significantly greater than the mid-parent heterosis (H_{MP}) for NLB resistance for the two BC hybrid populations (-357.8 and -361.3). Moreover, H_{MP} for the two BCF_1 populations was negative (Table 1).

Significant correlations were observed between average disease scores of IBMDHLs and their BC hybrids (BC_{B73}- 66% and BC_{Mo17}- 57%, $p<0.0001$). Additionally, IBMDHLs were significantly correlated with the mid-parent heterosis for NLB resistance of BC_{B73} hybrids and BC_{Mo17} hybrids (45% and 79%, $p<0.0001$). Very low correlations were observed between the BC

hybrid populations and their mid-parent performance (H_{MPB73} - 37% and H_{MPMo17} - 5%) (Table 2).

QTL analysis and gene actions of QTLs associated with NLB resistance in IBMDH and BC hybrid populations

Average AUDPC values for the IBMDH, BC_{Mo17} and BC_{B73} populations were used for QTL analysis. QTLs associated with NLB resistance were detected in all three populations at an experiment-wise significance threshold of $\alpha = 0.05$. Significance of a marker was set at a logarithm of odds (LOD) score of 2.4.

A total of 26 NLB resistance-associated QTLs were identified, coming from all chromosomes except chromosome 7. QTL effect magnitudes varied in the range of approximately 48.6 and 190 AUDPC trait units (Table 3). Thirteen NLB resistance-related QTLs with additive effect magnitudes of 39 to 189 were detected in either the IBMDH population or BC hybrid populations. Six of these QTLs increased disease severity by 71.6 – 189.2 AUDPC units and explained 1.43% to 14.23% of the phenotypic variation. The remaining seven QTLs explained 1.62% - 9.96% of the phenotypic variation reducing disease severity by 76.1 to 177.1 AUDPC units. Three QTLs with dominant gene effect magnitudes ranging from 139.7 to 273.6 overlapped in the IBMDH, BC_{B73} and H_{MPMo17} or IBMDH and H_{MPMo17} or IBMDH and BC_{B73} populations. The overlapping QTLs between IBMDH and BC_{B73} populations increased disease severity by 160.1-176.0 AUDPC units and explained 6.57% to 8.69% of the phenotypic variance (Table 3). Two of the three overlapping QTLs had opposite effects to NLB resistance, i.e. increased disease severity in one population (positive effect) and decreased resistance in another population (negative effect). Additionally, 0.96% - 8.66% of the phenotypic variation detected in H_{MP} was explained by 10 over-dominant effect QTLs, eight of which increased disease by 48.6-145.5 while two decreased disease severity by 104.7-134.7 AUDPC trait units.

Discussion

IBMDH and two F_1 backcross hybrid (BC_{B73} and BC_{Mo17}) populations were used to study genetic effects of quantitative disease resistance. BC_{F1} populations are unique in that DHLs with fixed genotypes are crossed to a recurrent parent of the DHLs, B73 or Mo17 creating BC_{B73} and BC_{Mo17} populations, respectively. This cross resulted in BCF_1 with known marker genotype i.e. homozygote if the DH is crossed to a recurrent parent with the same marker allele or heterozygote if DH is crossed to a recurrent parent with opposing alleles. Because the genotypes of these DHLs are fixed, studies are easily replicated over time by simply crossing DH lines with genotypes of interest to develop research populations.

The two back cross populations were more resistant to NLB than the IBMDH population. The differences in NLB severity scores across IBMDH, BC_{B73} and BC_{Mo17} populations were supported by differences in QTLs identified in these three populations. QTLs related to NLB were identified in 26 confidence interval regions distributed throughout 9 of the 10 maize chromosomes. QTLs were identified across 8 regions on chromosome 1, one region on chromosome 2, 5 and 10, three regions on chromosome 3, 4, 6, 8 and 9 (Table 3). Nine of the 26 confidence interval regions consisted of QTLs detected exclusively in BC_{F1}. It is possible that QDR acts in single copies since introduction of heterozygosity through backcrossing resulted in detection of QTLs. Furthermore, the higher number of additive QTLs in the two BC_{F1} populations correlates to the significant reduction in disease severity in the two BC_{F1} populations demonstrated in Figures 2 and 3.

Genetic effects were derived as: $a = (P_1P_1 - P_2P_2)/2$, $H_{MP} = d = (BC_{F1} - MP)$ where $MP = (P_1P_1 + P_2P_2)/2$ and $BC_{F1} = (a + d)$ or $(a + d + P_2P_2)$ depending on whether P_2 or P_1 was the recurrent parent while maintaining P_1P_1 as the BC_{F1} genotype (Jiang et al. 2014). Mid parent

heterosis for NLB resistance was calculated to estimate performance of the BC_{F1} hybrids. Moreover evaluation of IBMDH, BC_{F1} populations and mid-parent heterosis was used to determine gene actions for identified loci. Additive, dominant, over-dominant and under-dominant effects were assessed and reported in this study. QTLs were considered additive when identified exclusively in IBMDH or BC_{F1} populations and dominant when identified across IBMDH, BC_{F1} and H_{MP}. Over-dominant effect QTLs were exclusively detected in H_{MP}.

The observed significant correlation between IBMDHLs and BC_{F1} hybrids (Table 2) suggests strong additive gene action at QTLs that underlie NLB resistance. The 10 QTLs identified under mid-parent heterosis showed over-dominance genetic effects in BC_{B73} and BC_{Mo17} populations whereas only three QTLs showed complete or partial dominance genetic effects. Similarly, (Jiang et al. 2014) assessed M-QTL and E-QTL associated with growth traits in rice DH and two BC_{F1} populations. (Jiang et al. 2014) reported a higher number of QTLs exhibiting over-dominance gene effects than complete or partial-dominance effects. Furthermore, (Li et al. 2001; Luo et al. 2001)) and Mei et al., (2004) evaluated recombinant inbred lines, BC_{F1} and test cross populations to determine gene actions of QTLs underlying agronomic traits in rice. A higher number of over-dominance effect QTLs was reported in both studies demonstrating control of over-dominance on heterosis. Results from our study and previous literature confirm over-dominance as the major genetic effect impacting heterosis for quantitatively controlled traits including disease resistance. Hence single copy action of QTL and over-dominance genetic effects should be given great attention when selecting favorable allelic combinations in maize breeding programs.

Figures and Tables

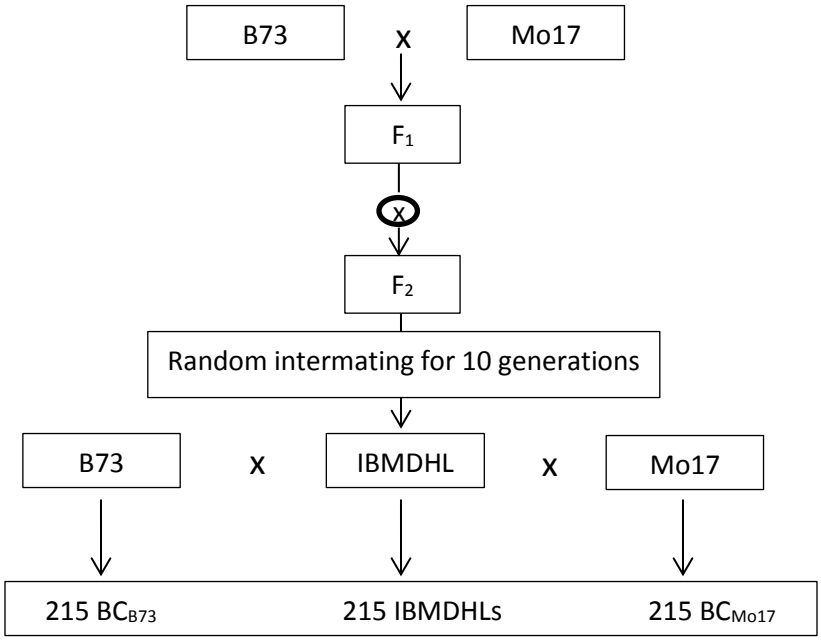

Figure 1: Diagrammatic representation of IBMDHL and BC hybrid population development

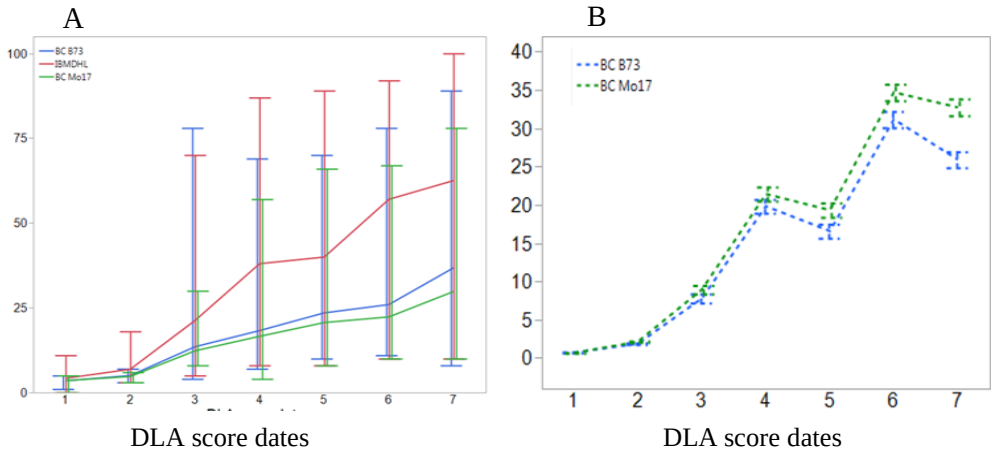

Figure 2: Differential disease progression for the IBMDHLs versus the two backcross populations. **A.** AUDPC means and standard errors are reported for the three populations, revealing the disparity between inbred and hybrid levels of disease resistance. **B.** Comparative resistance of backcross hybrid populations as revealed by the difference in AUDPC values between the IBMDHLs and their corresponding sets of backcross hybrids.

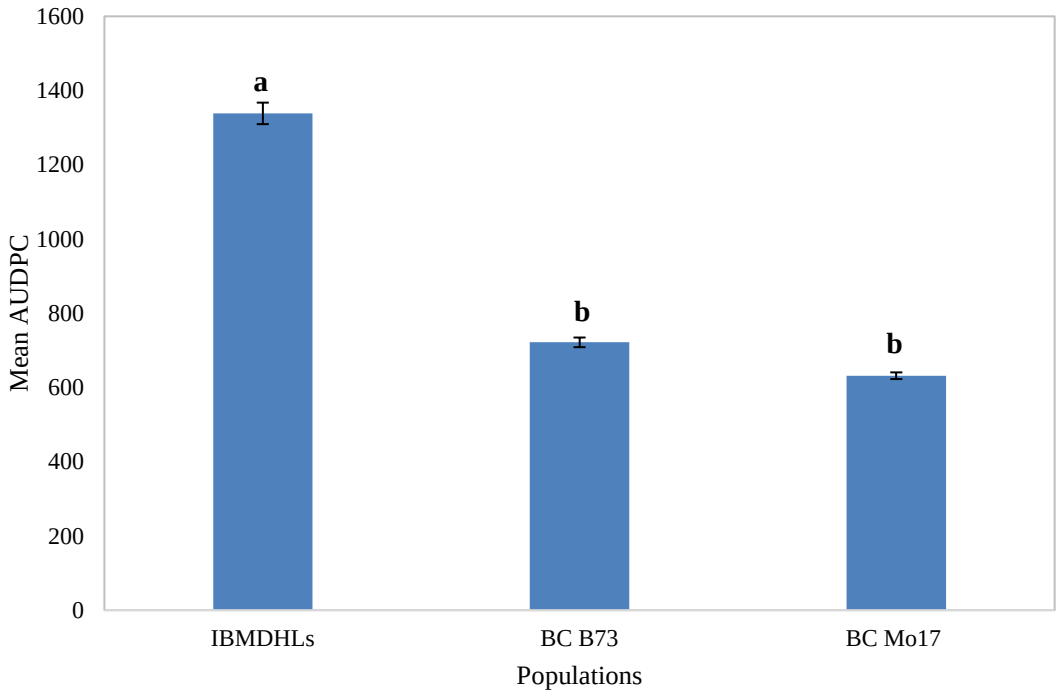

Figure 3: Average AUDPC values across IBMDHLs and backcross hybrids. a and b are significantly different at $p < 0.001$ by LSD

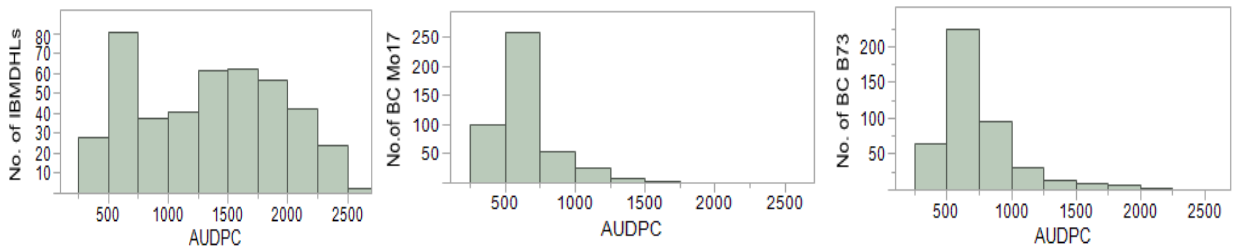

Figure 4: Distribution of mean AUDPC across IBMDHLs and BC hybrids in Ames, IA (2015)

Table 1: Statistical analysis of mean AUDPC of IBMDHLs and their backcross hybrid populations

	B73	Mo17	IBMDHs
Mean	928.7±5.4	752.3±125.0	1275.1±50.0
H_{MP}	-357.8±20.1	-361.3±20.5	
HB			434.6±50.0
B73		511.9±14.6	744.0±24.0
Mo17	515.2±22.8		652.4±15.3

AUDPC values are represented as means ± standard deviation. Mid-parental heterosis for disease resistance, H_{MP} = BC_{F1} – MP. MP represents mid-parent AUDPC for BC_{B73} hybrid ((IBMDHL+B73)/2) and BC_{Mo17} hybrid ((IBMDHL+Mo17)/2). Hybrid breakdown of the mid-parental value of B73 and Mo17, the two parental lines, HB = IBMDHL – MP where MP = (B73+Mo17)/2.

Table 2: Pearson correlation and coefficient of determination for mean AUDPC between IBMDHLs, the two BC_{F1} populations and their corresponding mid-parent performance in Ames, IA (2015).

	BC_{B73}		BC_{Mo17}		H_{MPB73}		H_{MPMo17}	
	r	R^2	r	R^2	r	R^2	r	R^2
IBMDH	0.66***	0.44	0.57***	0.33	0.45***	0.20	0.79***	0.62
H_{MPB73}	0.37***	0.14						
H_{MPMo17}			0.05	0.00				

AUDPC values are represented as means ± standard deviation. Mid-parental heterosis for disease resistance, H_{MP} = BC_{F1} – MP. MP represents mid-parent AUDPC for B73XMo17 ((B73+Mo17)/2), BC_{B73} hybrid ((IBMDHL+B73)/2) and BC_{Mo17} hybrid ((IBMDHL+Mo17)/2. Significant at ***p < 0.001

Table 3: M-QTL associated with Northern Corn Leaf Blight resistance in IBMDHLs, BC_{Mo17} and BC_{B73} their backcross hybrid populations

[a]Chromosome	[b]Flanking markers	[c]Genetic marker interval	[d]IBMDH Lines			[e]BC_{Mo17}			[f]HMP(Mo17)			[g]BC_{B73}			[h]HMP(B73)		
		(cM)	LOD	a	R2	LOD	a + d	R2	LOD	d	R2	LOD	a + d	R2	LOD	d	R2
1	SYN7082-SYN34616	80-83	3.5	189.2	9.7												
1	PZE-101059784-PZE-101065552	86-90										3.5	144.1	5.6			
1	SYN2804-PZE-101126936	115-118							4.0	128.9	6.4						
1	PZE-101128354-PZE-101134383	119-122													3.6	145.5	8.7
1	SYN35722-PZE-101145571	128-133	4.1	190.0	9.8				4.2	-165.6	10.8	9.9	273.6	21.6			
1	PZE-101152132-SYN39118	140-150													2.4	84.9	3.0
1	PZE-101227723-SYN27285	218-221				2.8	-123.7	10.0									
1	PZE-101241854-SYN20362	231-235										3.9	-177.1	8.5			
2	SYN21950-SYN14633	205-208							2.6	79.5	2.4						
3	SYN37496-PZE-103053660	79-84													3.3	109.7	4.6
3	PZE-103132614-SYN627	137-139				7.6	143.2	14.2									
3	PZE-103178060-SYN29674	199-207	2.6	71.6	1.4												
4	PZE-104100589-SYN15400	118-125	2.4	-94.1	2.4												
4	PZE-104112214-PZE-104114952	137-141				3.5	127.1	10.1									

Table 3: Continued

4	PZE-104146988-SYN23993	164-168							2.9	-134.7	6.7						
5	PZE-105154136-SYN21851	127-143													2.7	70.6	2.0
6	PZE-106042027-SYN2959	33-36	2.6	-76.1	1.6												
6	SYN24540-PZE-SYN12697	125-131							3.8	-106.2	4.3				2.8	-104.7	4.0
6	SYN10686-PZE-106126296	136-140													5.0	130.5	6.7
8	SYN36558-PZE-108011830	34-38	2.6	-119.8	3.8												
8	PZE-108042796-PZE-108054497	61-68	2.7	176.0	8.7							3.7	160.1	6.6			
8	SYN32177-PZE-108118398	120-130													3.6	48.6	1.0
9	PZE-109000243	0										2.7	78.8	1.7			
9	SYN3709-PZE-109013469	26-42	2.7	-130.1	4.4												
9	PZE-109082140-SYN38163	83-89	3.5	-181.2	8.3				3.3	139.7	7.5						
10	ZM009290-0678-SYN12667	109-113										2.9	-132.6	4.9			

[a]Chromosome on which the QTL is located

[b]Flanking markers of the 2-LOD interval within which the QTL lies

[c]CentiMorgan (cM) positions of the flanking markers of the interval within which the highest likelihood for the QTL lies

[d]Traits for QTL analysis were; mean AUDPC values for IBMDHLs, BC_{B73} and BC_{Mo17}, and calculated $H_{MP\ for}$ BC_{B73} and BC_{Mo17}

[e]The logarithm of odds (LOD) score of the position of maximum likelihood of the QTL in each trait.

[f]Expected gene effects of QTL in each trait. Additive (a) effects estimated from IBMDHLs, additive and dominant (a + d) effects estimated from BC_{F1}, and dominant effects from H_{MP}.

[g]R^2 - estimate of phenotypic variance (%) explained by individual QTL

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CHAPTER 5. GENERAL CONCLUSIONS AND PERSPECTIVES

Diseases are one of the major causes of yield losses in crops around the world with the extent of loss dependent on environmental conditions, presence or absence of the host and presence or absence of the disease causing pathogens (Nelson et al., 2018). Northern corn leaf blight is among the most notorious maize pathogens leading to approximately 50% yield losses in maize (Welz & Geiger, 2000, Degefu et al., 2004, Ferguson & Carson, 2004, Pratt & Gordon, 2006, Ferguson & Carson, 2007, Martin et al., 2011, Poland et al., 2011, Zhang et al., 2012). Crop diseases are generally controlled biologically (Sartori et al., 2015), culturally (Howard, 1996) and chemically using fungicides (Nelson et al., 2018). However, host plant resistance is the most effective means of reducing crop losses caused by disease (Mundt, 2014).

Plants prevent diseases qualitatively using resistant genes (R-genes), and/or quantitatively using minor genes (Kushalappa et al., 2016, St Clair, 2010). The hypersensitive response in qualitative resistance is induced by single race-specific major genes conferring complete resistance mainly for biotrophic pathogens (Flor, 1971, Giraldo & Valent, 2013, Kushalappa et al., 2016). Considerable research has gone into understanding the mechanisms underlying qualitative resistance and as a result several qualitative genes have been identified and bred for using techniques such as fine mapping, mutation and positional cloning (Jones & Dangl, 2006, Nelson et al., 2018). Qualitative genes eventually lose their effectiveness as a result of environmental changes (Galiano-Carneiro & Miedaner, 2017), and evolution of plant-pathogen interactions in which pathogens overcome plant resistance unless the R-gene target is a vital virulence or survival pathogen effector (Nelson et al., 2018, Carson & Vandyke, 1994). Quantitative disease resistance (QDR) on the other hand is more durable due to the difficulty in overcoming multiple resistant genes. Moreover, QDR is less affected by changes in the

environment (Carson & Vandyke, 1994) and is race non-specific (Galiano-Carneiro & Miedaner, 2017). Progress has been made in unravelling the genetic architecture of QDR in various plant-pathogen interaction systems (St Clair, 2010). However, understanding the mechanisms of genes that control QDR is complicated by the multigenic nature of QDR and the fact that these genes are of minor effect.

Environment not only affects pathogen growth, pathogen reproduction, crop invasion and disease development, but also quantitatively controlled phenotypic variation in severity of disease. Environmental effects on QTLs can be seen as presence or absence of QTLs in certain environments or as a degree of expression of the QTL evidenced by variation in level of resistance (Ma et al., 2006). Environmental impact on QDR loci was demonstrated in our multi-environment/isolate study by the wide phenotypic variations in northern corn leaf blight (NLB) severity across genotypes tested within and across locations and years. Analysis of variance (ANOVA) results showed statistically significant environment and genotype-by-environment effects on NLB resistance. Moreover, 18 QTL were uniquely identified in either Aurora 2011 or 2012, or Boone 2014 or 2015. Several studies have looked at genotype-by-environment interactions in order to determine stability of resistance against pathogens across multiple environments (Capettini et al., 2003, Paillard et al., 2004, Ma et al., 2006).

Recent research in Arabidopsis (Diener & Ausubel, 2005, Cole & Diener, 2013, Roux et al., 2014), rice (Fu et al., 2011, Fukuoka et al., 2014), maize (Zuo et al., 2015, Yang et al., 2017), wheat (Moore et al., 2015, Rawat et al., 2016) and soybean (Cook et al., 2012, Liu et al., 2012) has made use of fine mapping, transgenics, mutants, gene silencing, and comparative genomic techniques to identify minor effect genes underlying QDR (Nelson et al., 2018). Using QTL analysis, our multi-environment (two NLB isolates) study identified a total of 23 QTL among the

Intermated B73 x Mo17 doubled-haploid lines (IBMDHLs). 18 of the 23 QTL were expressed against individual *Setosphaeria turcica* isolates in individual years in Boone or Aurora while 5 of the 23 overlapped between locations and isolates. Furthermore, a total of 26 QTL in the genetic effect study were identified across IBMDH, $BC_{B73,}$ and BC_{Mo17} populations and mid-parent heterosis for NLB resistance (H_{MP}). Six QTL were identified uniquely in IBMDH lines, 7 in the BC_{F1} populations, 10 in H_{MP} and 3 overlapped across IBMDH, BC_{F1} populations and H_{MP}. Similarly, Robertson-Hoyt et al., (2006), Mukherjee et al., (2013) and Li et al., (2018) reported differential expression of QTL across genotypes in different environments. Variation in the number and type of QTL identified in our studies versus previous studies could have been brought about by differences in the environments where these studies were conducted, differences in the maize populations, as well as differences in the NLB isolates. Because minor genes are reported to be more affected by the environment than qualitative major genes (Ma et al., 2006), crop populations should be tested under multiple environmental conditions to select QTL that are stable across environments. Isolate specificity of these QDR genes should also be tested by screening crop populations against multiple NLB fungal isolates under the same environmental conditions.

Breeding for durable resistance is also achieved through development of genetically diverse populations that provide multiple forms of resistance, like a combination of qualitative and quantitative resistance (Nelson et al., 2018). Recombinant inbred lines (RILs) (Li et al., 2010), F_7 (Xiao et al., 1995), nested association mapping (NAM) (Kump et al., 2011) and Intermated B73 x Mo17 (IBM) populations (Sharopova et al., 2002, Hussain et al., 2007) provide diverse allelic combinations making it difficult for pathogens to overcome resistance. Furthermore, breeders have developed superior populations using polycross, diallel and NC-

Design I, II, III mating designs (Hallauer et al., 2010). The population used in the multi-environment study was developed through double haploidization of IBM lines that had undergone 10 generations of intermating at the F_2 stage. Intermating increased cross over events which in turn provided us with a high marker density map and high resolution for identification of QTLs (Hussain et al., 2007). The two back cross populations in the genetic effect study were developed by crossing the IBMDH population back to its two parental lines, B73 and Mo17. These populations yielded novel NLB resistance-related QTL. Additionally, QTL that co-localized with previously reported qualitative or quantitative disease resistance genes were identified using our populations. This emphasized the diversity in resistance mechanisms offered by these 3 populations.

Backcross hybrid populations are a unique resource for studying gene effects such as additive, dominant and epistatic interactions (Acquaah, 2007, Jiang et al., 2014). Information about gene effects and the genetic variances underlying QTL directs decisions for selecting superior parental populations, predicting performance of hybrids, and predicting response to selection (Bernardo, 2002). Our genetic effect study used IBMDH lines and their backcross hybrid populations to identify QDR gene actions and understand how these gene actions are impacted by different genetic backgrounds. Assessing the two back cross hybrid populations against the IBMDH population revealed sets of QTL unique to IBMDH, BC_{B73} and BC_{Mo17} populations, and a set of QTL that overlapped across the three populations. Some of these QTLs showed additive, dominant or over-dominant effects. Genes located in 13 QTL demonstrated additive effects; 6 of which were unique to IBMDH and 7 to BC_{B73} or BC_{Mo17} populations. Ten QTL with over-dominant genetic effects were identified solely in H_{MP} of BC_{B73} or $BC_{Mo17.}$ Three dominant effect QTL overlapped across IBMDH, BC_{F1} populations and H_{MP}.

Identification of useful QTL is highly dependent on reliable and comprehensive physical and genetic maps. The maize genome, among several other genomes has been sequenced and annotated (Jiao et al., 2017). Genetic and genomic approaches such as bacterial artificial chromosome (BAC-by-BAC) and PacBio Single Molecular Real Time (SMRT) were used to sequence the maize B73 genome. The B73 genome went through stages of ordering and orientation of physical contigs from B73 RefGen_V1 (Schnable et al., 2009, Wei et al., 2005) to B73 RefGen_V2 to B73 RefGen_V3 (Law et al., 2015) to the current B73 Reference genome V4 (Jiao et al., 2017). Our study integrated a new B73 physical map with a high resolution IBMSny10DHL population in order to anchor unanchored contigs and expand our understanding of the causes of QTL variations occurring in germplasm.

In a nutshell, genetically diverse populations are great resources that can be used to construct comprehensive physical and genetic maps from which variant QTLs are identified. Identified QTLs should be tested for consistency across multiple environmental conditions. Stacking these QTLs in individual germplasm will provide populations with durable resistance. The ultimate goal for breeders is to increase yield. Therefore, care should be taken to avoid sacrificing yield for resistance.

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